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REVISION OF ALLOCREADIOIDEA NICOLL, 1934. PART—I. FAMILIES : LEPOCREADIIDAE NICOLL, 1934, DEROPRISTIIDAE N. FAM., HOMALOMETRIDAE N. FAM. AND MASENIIDAE GUPTA, 1953.

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Cable and Hunninen (1942) created the subfamily Depropristiinae for *Depropristis* Odhner, 1902 and Homalometrinae syn. Anallocreadiinae Hunter and Bangham, 1932 for *Homalometra* Stafford, 1904 and *Microcreadium* Simer, 1929 on the basis of their study of the life-cycle of *Depropristis inflata* and morphological features of their adults. They separated the two new subfamilies from the Lepocreadiinae Odhner, 1905 and included them in the Lepocreadiidae Nicoll, 1934 on account of the cercariae of trichocercous type developing in simple rediae in gastropods and encysting into metacercariae in invertebrates.

The life cycle of *Stephanostomum tenue* (Linton), a member of Acanthocolpidae as demonstrated by Martin (1939) also revealed that the Acanthocolpidae due to their true opthalmoxiphidiocercariae with simple spear shaped stylet and a tail shorter than body, long prepharynx. subglobular pharynx and short caeca not extending behind acetabulum, Y shaped excretory bladder with stenostomate protonephridae differ much from the Depropristiinae, Homalometrinae and Lepocreadiinae characterised by their trichocercous cercariae. The Acanthocolpidae also differ in that their opthalmoxiphidiocercariae utilize small fish as their second intermediate host instead of invertebrates in which they become encysted into metacercariae. Thus the life cycle of Acanthocolpidae Lühe differs remarkably from that of Lepocreadiidae Nicoll.

We separate Depropristiinae Cable and Hunninen, 1942 and Homalometrinae Cable and Hunninen, 1942 from the Lepocreadiidae and elevate them to the rank of Depropristiidae n. fam. and Homalometridae n. fam. as they differ from the Lepocreadiinae Odhner, 1905 characterised by setiferous tailed cercariae possessing two conspecuous eyes and a long tail much longer than body bearing lateral tufts of setae in transverse rows on either side. The cercariae (*Lepocreadium setiferous* Miller and Northup) which develop in rediae in marine gastropods and

cheyst in marine invertebrates lack a stylet possess two suckers, a very short pharynx, short oesophagus, cephalic glands and irregularly scattered cystogenous glands, tubular or saccular excretory vesicle with mesostomate protonephridia such as is met with in the typically Allocreadiid genera. Such excretory system also resembles that of the trichocercous cercariae of Dercpristiinae Cable and Hunninen and Homalometrinae Cable and Hunninen.

Cable (1952) created the genus *Pristicola* for *Dihemistephanus sturionis* Little, 1930 as the genotype and only species and also created the genus *Pristotrema* with *Pristotrema manteri* Cable, 1952 as the genotype and only species under the subfamily Dercpristiinae. According to him *Pristotrema* stands intermediate between *Dercpristis* and *Pristicola* in the extent of vitellaria and uterus. As the spination of the cirrus and metraterm of the three genera is of a different kind from that in the Acanthocolpidae and as they all occur in sturgeons it "emphasises their close relationship and points to a parallel evolution of parasite and host" and suggests their inclusion in the Dercpristiinae Cable and Hunninen, 1942. He pointed out "should the precedent set by other investigators in erecting families for small groups on the basis of adult structure be followed, the Dercpristiine would be elevated to family rank." But he finally decided to maintain it in the family Lepocreadiidae. He thus admitted the uncertain family position of the Dercpristiinae and did not remove it from the Lepocreadiidae.

Yamaguti (1958) has again placed the Dercpristiinae in the family Acanthocolpidae. He created the subfamily Pristicolinae Yamaguti, 1958 for the genus *Pristicola* Cable, 1952 under the Acanthocolpidae. We include Pristicolinae Yamaguti, 1958 in the Dercpristiidae n. fam., which thus includes subfamilies Dercpristiinae Cable and Hunninen and Pristicolinae Yamaguti.

Trichocercous cercariae

The term "Trichocercous cercariae" has been very vaguely in use. It is a heterogenous group of larvae which may be divided into three subdivisions : (1) trichocercous cercariae of Fellodistomatoidea, better regarded as setiferous tailed cercariae (family Fellodistomatiidae), (2) Setiferous tailed cercariae of Lepocreadiidae Nicoll, and (3) trichocercous cercariae of Dercpristiidae n. fam., Homalometridae n. fam. which may preferably be called "Anallocreadine" cercariae after Hopkins, possessing tails with 3-6 pairs of ventro-lateral tubercles each with a delicate "Hair" obviously sensory and not locomotor in function unlike the bristles or setae of the setiferous tailed cercariae. Though the latter may be considered as a larval adaptation, they have a phylogenetic significance in that they have arisen in two remotely related groups of distomes such as the Fellodistomid stock and the Allocreadiids of the family Lepocreadiidae. The setiferus tailed cercariae (erroneously called trichocercous cercariae mentioned under subdivision (1) of Fellodistomatidae are characterised by the presence of U, V or Y shaped excretory vesicle with stenostomate protonephridia, in contrast to that of the setiferus tailed cercariae of the Lepocreadiidae which have their excretory system of tubular or saccular excretory vesicle with mesostomate protonephridia such as is met with in the typically Allocreadiid genera and also in Dercpristiidae n. fam. and Homalometridae n. fam.

Dercpristiidae n. fam.

Diagnosis.—Allocreadioidea Nicoll. Cuticle with extremely prominent spines decreasing gradually in size and number towards posterior end. Remnants of eye spots usually present. Prepharynx short or absent ; oesophagus short, genital pore midventral in front of acetabulum. Ovary pretesticular separated from

anterior testis by uterine coils. Testes tandem or slightly diagonal in postetior half of body. Cirrus sac well developed, claviform, extending behind acetabulum. Vesicula seminalis bipartite with intervening transverse septum or sphincter, anterior chamber concerned with spermatophore formation. Receptaculum seminis and Laurer's canal present adjacent to ovary. Cirrus and metraterm armed with prominent spines. Pars prostatica weakly developed with inconspicuous prostate cells. Genital atrium short or moderately long, tubular and unarmed. Vitellaria not extensively developed with rather scanty vitelline follicles begining behind acetabulum at the level of vesicula seminalis and not extending into extreme posterior region of body and not occupying entire post-testicular region. Uterus much coiled, extending from ovary first posteriorly to or beyond testes and then anteriorly to join metraterm. Eggs numerous, medium in size and operculate. Excretory vesicle short, tubular or sac like with mesostomate protonephridia. Cercariae trichocercous with eyes and simple tail with a ventral finfold bearing ordinarily six pairs of ventrolateral tubercles each with a delicate "hair" and without a true stylet, developing in rediae in prosobranch gastropods and encysting in invertebrates as a second intermediate host. Adults parasitic in intestine of migratory fishes mainly eels and sturgeons.

Type genus : *Deropristis* Odhner, 1902.

Subfamily Deropristiinae Cable and Hunninen, 1942.

Subfamily diagnosis.—Deropristiidae n. fam. Cervical expansions present or absent. Circumoral crown of spines absent. Oral sucker not conspicuously larger than ventral sucker. Prepharynx short or absent. Testes directly tandem or separated from one another by uterine coils. Ovary in middle third of body much in front of testes and separated from anterior testis by uterine coils. Vitellaria lateral, vitelline follicles rather scanty confined to middle region of body terminating at level of hinder margin of anterior testis or much in front of it.

Type genus : *Deropristis* Odhner, 1902.

Other genera : *Pristotrema* Cable, 1952, and *Skrjabinopsolus* Ivanov, 1935.

Subfamily Pristicolinae Yamaguti, 1958.

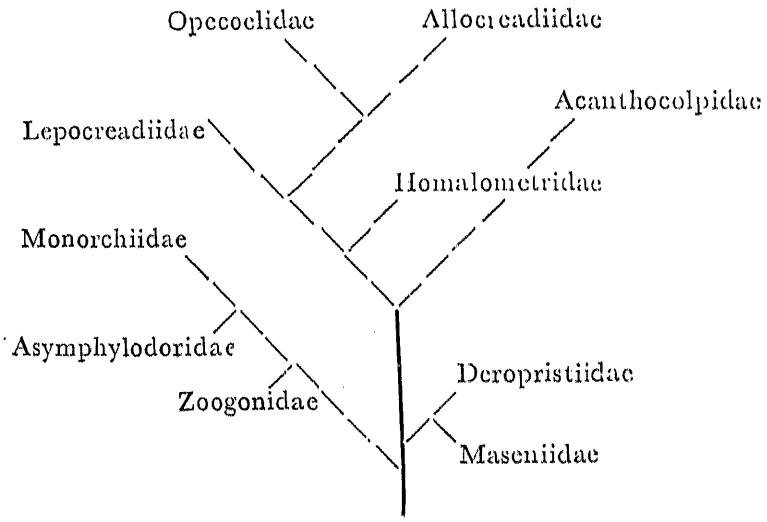
Subfamily diagnosis.—Deropristiidae n. fam. Spines in forebody anteriorly becoming much smaller except for the double row of flat circumoral spines. Circumoral crown of spines in two rows with a ventral gap present. Oral sucker larger than ventral sucker. Prepharynx and oesophagus short. Testes tandem, slightly separated near posterior end. Ovary much behind middle of body a little in front of anterior testis. Vitellaria extending laterally and dorsally from vesicula seminalis to slightly behind testes but not reaching extreme posterior region and not occupying entire post-testicular region.

Type genus : *Pristicola* Cable, 1952.

The subfamily Homalometrinae Cable and Hunninen, 1942 (syn. Anallocreadinae Hunter and Bangham, 1932) created by the latter authors under the Lepocreadiidae Nicoll is also excluded from the latter family and raised to the rank of the family Homalometridae n. fam. as it differs on account of its trichocercous cercaria having short tubular or sac-shaped excretory vesicle with mesostomate protonephridia. The cercaria lacks a true stylet, but possesses a papilla like structure, which is in all probability a rudimentary stylet. Cable and Hunninen think the cercaria of *D. inflata* is an intermediate form between the opthalmoxiphidiocercariae and trichocercariae.

We have elevated Homalometrinae to the rank of the family Homalometridae n. fam. as it differs fundamentally from the Lepocreadiidae Nicoll in the character of its trichocercous cercariae as discussed before. Homalometridae n. fam. is also created to indicate the striking differences from the Lepocreadiidae Nicoll, Deropristiidae n. fam. and Allocreadiidae Stossich in the morphology of the adult.

The Acanthocolpidae is a primitive family of the Allocreadioidea. It appears that evolution took place along divergent lines from the Echinostomid ancestor (Superfamily Echinostomatoidea) giving the family Acanthocolpidae as one main branch, Deropristiidae n. fam. another small branch near its origin and the third main branch,—Lepocreadiidae branch with its branches, the Lepocreadiidae, Homalometridae as a short twig near its base close to the Deropristiidae branch and the larger twig, giving the families Allocreadiidae Stossich and Opecoelidae Ozaki as shown in the schematic phylogenetic tree. The family Monorchiidae evolved separately from the Echinostomid ancestor as primitive stock with Asymphylodoridae n. fam. and Zoogonidae Odhner, 1911 as its branches, represents the fourth main stem of the superfamily.



Schematic phylogenetic tree of superfamily Allocreadioidea

Homalometridae n. fam.

Family diagnosis.—Allocreadioidea. Body spinulate. Prepharynx present or absent ; oesophagus short or absent ; Acetabulum in anterior half of body or almost equatorial. Genital opening median or submedian immediately in front of acetabulum or behind intestinal bifurcation. Testes tandem, oblique or parallel, equatorial or postequatorial. Cirrus sac short, anterolateral or immediately anterior to acetabulum or absent. Ovary pre-testicular. Receptaculum seminis and Laurer's canal present. Vitellaria well developed, lateral extending from pharynx or intestinal bifurcation to hinder end and confluent in post-testicular region or entirely restricted to and filling post-testicular region. Uterus between testes or anterior testis and acetabulum. Ova a few or small in number. Cercariae trichocercous ("Anallocreadine") without stylet, with eyes, well developed prepharynx, pharynx, oesophagus shorter than pharynx and caeca extending

behind acetabulum. Excretory vesicle rectangular, sac like, tubular or sausage-shaped with mesostomate protonephridia, and straight slender tail bearing three or more pairs of papillae with a seta or sensitive hair and sometime very narrow dorsal and ventral fins. Cercariae develop in fresh water and marine proso branch gastropods. Adults parasitic in fresh water and marine fishes.

Type genus : *Homalometron* Stafford, 1904 (syn. *Allocreadium* Simer, 1929)

Subfamily Homalometrinae Cable and Hunninen, 1942.

Subfamily diagnosis.—Homalometridae n. fam. Body oval or fusiform, not very elongate. Prepharynx present ; oesophagus short. Genital opening immediately preacetabular or immediately behind intestinal bifurcation. Testes tandem or oblique, postequatorial. Cirrus sac absent and vesicula seminalis free in parenchyma. Vitellaria lateral extending from level of intestinal bifurcation to hinder end, confluent in post testicular region. Uterus between anterior testis and acetabulum. Ova small in number. Excretory vesicle tubular reaching posterior testis or ovary. Adult parasitic in intestine of fresh water and marine fishes.

Type genus *Homalometron* Stafford, 1904.

Other genera included are *Crassicutis* Manter, 1936 and *Pancreadium* Manter, 1954.

Subfamily Microcreadiiane n. subfam.

Subfamily diagnosis.—Homalometridae n. fam. Body small, somewhat plump. Oral sucker large. Prepharynx present or absent. Oesophagus absent. Testes parallel side by side, equatorial or tandem postequatorial. Cirrus sac short, anterodorsal to acetabulum or immediately preacetabular. Vitellaria post-equatorial, united, entirely restricted to and filling post-esticular region or extending from pharynx to hinder end. Uterus between testes and acetabulum. Ova a few only. Excretory vesicle tubular reaching behind testes. Parasitic in intestine of fresh water fishes.

Type genus : *Microcreadium* Simer, 1929.

Other genus included is *Procreadium* n.g. with the genotype and only species *Procreadium ictaluri* syn. *Allocreadium ictaluri* Pearse, 1924.

A new subfamily Assamiinae is created under the family Homalometridae n. fam. to accommodate *Assamia gauhatiensis* Gupta, 1953. The genus *Assamia* Gupta, 1953 does not belong to the family Opisthorchidae Braun, 1901 in which the author has included it, as the vitellaria are intercaecal and post-acetabular in position and the uterus is short, forming a few coils between the testes and genital pore. The excretory bladder is tubular extending upto the hinder region of ovary. The genus belongs to the family Homalometridae n. fam. of the superfamily Allocreadioidea Nicoll. It connects the two subfamilies of the latter family, Homalometrinae Cable and Hunninen, 1942 and Microcreadiinae n. subf. In the absence of cirrus sac it resembles the subfamily Homalometrinae, whereas in the entirely postacetabular and postequatorial position of the vitellaria it resembles the subfamily Microcreadiinae n. subf. The uterus lies between testes and acetabulum containing a few ova. It, therefore, becomes feasible to create a new subfamily Assamiinae under the Homalometridae n. fam. to accommodate *Assamia* Gupta which has non-spinate bodywall and prepharynx is absent. The oesophagus possesses a muscular oesophageal pouch at its posterior end and the two caeca arise from the dorsal side of the oesophageal pouch. The genital pore lies at about half way between

oesophageal pouch and acetabulum. The genital atrium has strongly muscular walls formed of strong sphincter muscles. The tubular excretory bladder has a vesicle like structure at its anterior end immediately behind acetabulum.

Assamiinae n. subf.

Subfamily diagnosis.—Family Homalometridae n.fam. Body fusiform, dorso-ventrally flattened with anterior broader and narrow posterior ends. Bodywall unspinate. Suckers well developed, Acetabulum larger than oral sucker pre-equatorial or subequatorial. Oral sucker large ; prepharynx absent ; pharynx well developed, longer than broad. Oesophagus short, thin walled produced posteriorly into a muscular pouch. Caeca narrow, arising dorsally from dorsal side of oesophageal pouch by a common opening and extending to a little distance in front of posterior extremity. Genital pore median, half way between oesophageal pouch and acetabulum. Genital atrium with strongly muscular walls of sphincter musculature. Testes intercaecal, almost symmetrical, postacetabular. Cirrus sac absent. Vesicula seminalis from behind acetabulum to genital pore. Ovary immediately postacetabular. Receptaculum seminis close to the side of ovary. Laurer's canal present. Vitellaria follicular, intercaecal and post-ovarian restricted to and filling post-testicular region terminating a little in front of hinder end of caeca. Uterus short forming a few coils, intercaecal between testes and genital atrium. Metraterm well developed. Excretory bladder tubular extending to ovary and swollen at anterior end near posterior end of ovary. Eggs non-operculate, a few. Parasitic in intestine of fresh water fishes.

Type and single genus : *Assamia*, Gupta, 1953. With only species *Assamia gauhatiensis* Gupta, 1953.

The family Maseniidae Gupta, 1953 is closely related to the subfamily Pristicolinae Yamaguti, 1958 of the family Deropristiidae n. fam. which it resembles in the oral sucker being larger than acetabulum and provided with the double row of flat circumoral spines with or without gap, short prepharynx and short oesophagus, tandem or obliquely tandem testes situated closely behind ovary, vesicula seminalis being bipartite with intervening transverse septum or sphincter, and vitellaria of restricted extent extending to testes. The excretory bladder is tubular or saccular extending to posterior testis. The two distinctive features of this family are the extremely anterior position of genital pore on the dorsal side of oral sucker and extremely long cirrus sac extending from anterior end to acetabulum or even a little behind it. There is no doubt that this family belongs to the superfamily Allocreadioidea and is closely related to the subfamily Pristicolinae of the Deropristiidae n. fam. and Acanthocolpidae. Price (1940) was correct in saying that the presence of a cirrus pouch suggests the affinities of Maseniinae Chatterji (1933) created for *Masenia collata* with the Acanthocolpidae Luhe, 1909.

Maseniidae Gupta, 1953

(syn. Maseniidae Yamaguti, 1953)

Family diagnosis.—Allocreadioidea : Cuticle spinulate. Oral sucker terminal, large, funnel shaped, much larger than acetabulum with circumoral interrupted or uninterrupted, middorsally double crown of spines. Acetabulum well apart from oral sucker, pre-equatorial at junction of anterior and middle third. Prepharynx and oesophagus short ; ceaca simple extending to a little distance in front of hinder end. Genital pore at extreme anterior end on dorsal side of oral sucker. Testes two, post acetabular almost tandem or obliquely tandem closely behind

ovary and in contact with each other. Cirrus sac extremely long, claviform and curved extending from anterior extremity to acetabular zone consisting of a basal saccular and distal tubular parts. Vesicula seminalis bipartite with intervening transverse septum or sphincter. Pars prostatica with prostate cells well developed. Ovary postacetabular. Receptaculum seminis and Laurer's canal adjacent to ovary. Vitellaria follicular, lateral, not extensive from acetabulum to testes. Uterus large post-testicular and pre-testicular. Excretory vesicle saccular or tubular. Parasitic in fresh water fishes.

Type genus : *Masenia* Chatterji, 1933.

Other genus is *Eumasenia* Srivastava, 1951.

Yamaguti recently (1958) has not recognized Lepocreadiidae Nicoll and treated it as subfamily Lepocreadiinae Odhner, 1903 under the family Allocreadiidae Stossich, 1903. He has also included Homalometrinae Cable and Hunninen, 1942 in the latter family. Hopkins (1941) remarked "I suspect that all Allocreadiid like genera with cotylocercous cercariae and 2 [(2+2)+(2+2)] flame cell formula form a natural taxonomic group, distinct from those with opthalmoxiphidiocercariae (*Crepidostomum* and its relatives), those with Trichocercous cercariae (Lepocreadiinae), and those with (Anallocreadine cercariae *Anallocreadium* and *Microcreadium*)." Cable and Hunninen thought that the closest relatives of the Acanthocolpids are to be sought among the Allocreadiids such as *Allocreadium, Crepidostomum* and *Megalogonia.* Yamaguti (1958) who has done a great service in producing a comprehensive compilatory work, has not taken into consideration the characters of cercariae and life cycle data in his scheme of classification. The criteria of morphological characters of the adult used by him in the diagnosis of the family are too wide and rather vague to be of much value as concieved at present. There is no doubt the Allocreadiidae, which is one of the largest family of the Digenea, is cumbersome and difficult to define in its scope unless it is split up into a number of well defined families on the basis of morphological features of the adult as well as of the cercariae and general life cycle. The morphological features should be as far as possible sharply distinctive so that their close affinities and those of the subfamilies under them can be clearly determined. Dawes (1956) in his key to subfamilies of Allocreadiidae gives :—

"I. Cuticle spinose (parasitic in marine fishes)..... ..... .Lepocreadiinae

II. Cuticle non-spinose (parasitic in marine and fresh water fishes)

A. In marine fishes (excepting*)..... . .. . .Allocrediinae

B. In fresh water fishes

(*a*) Oral sucker with anterior processes.....Stephanophialinae (*Crepidostomum.*)

(*b*) Oral sucker without anterior processes.....Sphaerostomatinae (*Sphaerostoma*)"

In regard to Lepocreadiinae, which we now conceive as family Lepocreadiidae the distinctive features are—Cuticle spinose, and parasitic in digestive tracts of marine fishes, exceptionally freshwater fishes. The other distinctive features are—Cirrus sac present enclosing vesicula seminalis interna, prostatic complex and cirrus, vesicula seminalis externa free or surrounded by prostatic cells in parenchyma present (except Postporinae Yamaguti, 1958, in which tubular coiled seminal vesicle lying free in parenchyma is present and cirrus sac is absent although tubular pars prostatica surrounded by a membrane is present) ; cercariae of Lepocreadiidae are setigiferous tailed *i.e.*, with tail much longer than body provided with lateral tufts of setae, and with conspicuous eye spots but without stylet, developing in

rediae without collar and ambulatory processes in marine snails ; metacercariae encysted or unencysted in inverteberates, triclad turbellarians, spinoid worms, medusae, ctenophores; excretory vesicle of cercaria tubular extending to acetabulum or beyond with mesostomate protonephridia ; excretory vesicle of metacercaria and adult often long and tubular extending to acetabulum and beyond, sometime even to pharynx (*Opechona* syn. *Pharyngora*), sometime to ovary behind acetabulum Petalocotylinae Ozaki, 1937, exceptionally short, reachin g to hinder end of posterior testis (Opisthogonoporinae Yamaguti, 1958).

Though the Lepocreadiinae is mostly parasitic in intestine of marine fishes, there are a few exceptional genera parasitic in fresh water fishes. The life cycles of only a few members of the Lepocreadiidae have been demonstrated. Palombi (1937) showed that *Lepocreadium album* Stossich possesses setiferous tail much longer than body with lateral tufts of setae, which develops in marine snails, and becomes encysted in nudibranchs. Martin (1938) traced the life cycle of *L. setiferoides* (Miller and Northup, 1926), which is very similar to that of *L. album*, possessing also a similar setiferous tailed cercaria developing in rediae in the marine snail *Nassa obsoleta*. The cercaria *Opechona bacillaris* is well known to have tail much longer than body with lateral tufts of setae.

Manter (1947) described several forms, *Lepocreadium inulla* (Linton, 1947) Linton, 1910, *Lep. bimarium* Manter, 1940, *Pseudocreadium anandrum* n. sp., *Opechona gracilis* (Linton, 1910) n. comb., *Homalometron elongatum* n. sp., *Crassicutis marina* n. sp., *Opisthoporus epinepheli* n. gen., n. sp. The latter genus named *Postporus* Manter, 1940 constitutes the subfamily Postporinae Yamaguti, 1958 under the Allocreadiidae. Another species of this genus *i.e.*, *Postporus mycteropercae* Manter (syn. *Opisthoporus mycteropercae* Manter, 1947) was also described. Manter added notes on four species of *Lepidapedon* and *Myzoxenus lachnolaimi* Manter, 1947. He also described *Bianium olicitum* Manter, 1931, *Multitestis chetodoni* Manter, 1947. In the discussion under the latter species he mentions that *Rhagorchis* is very closely related to *Multitestis* Manter, 1931. He also described *Enenterum aurium* Linton, 1910 under the Lepocreadiidae as set by Cable and Hunninen (1940), and in the discussion under it he mentions that he considers it as one of the latter family "in spite of its lack of eye-pigment even in young specimens. Perhaps it belongs to the Opecoelidae but it has a spiny cuticle, seminal vesicle and and large prostate gland."

Manter (1947) also accepts the family Opecoelidae Ozaki, 1925. He writes "Cable and Hunninen (1942 : 306) agree with Hopkins (1941 : 42–43) in such a restricted limitation of Allocreadiidae. Species known to possess cotylomicrocercous cercariae apparently fall into the family Opecoelidae, while the genera *Allocreadium* Looss, 1900 ; *Crepidostomum* Braun, 1900 ; *Megalogonia* Surber, 1928 ; *Bunodera* Railliet, 1896 ; and perhaps others remain in the Allocreadiidae." While we agree in regard to *Allocreadium Crepidostomum* and *Megalogonia* to be included in the Allocreadiidae, we accept the family Bunoderidae Nicoll, 1914 as *Cercaria nodulosa*, which is xiphidiocercaria of virgula group developing in sporocyst in *Plaudina impura* and encysts in the same host (Linstow, 1873), is sufficiently distinctive to permit of the status of a family for this group. Besides, the subfamily Bunoderinae Looss, 1902 has such characteristic morphological features in the adult that its status as a family would be amply justified. We, therefore, recognise and maintain the families, Lepocreadiidae Nicoll, Opecoelidae Ozaki, Allocreadiidae Stossich and Bunoderidae Nicoll. The new families Deropristiidae and Homalometridae have been already dealt with under superfamily Allocreadioidea Nicoll. The families Homalometridae and Allocreadiidae are closely related as the new genus *Procreadium* with the genotype *Procreadium ictaluri* (syn. *Allocreadium ictaluri* Pearse, 1924) stands as a connecting link between the two families.

Manter (1947) mentioned the diagnosis of the Opecoelidae "Body smooth, more or less elongate, usually flattened ; anus or ani may be present ; excretory vesicle I-shaped ; testes postovarian, usually two (nine in *Helicometrina*), tandem or diagonal ; cirrus sac present, lacking or weakly developed ; ovary pretesticular ; uterus usually entirely preovarian, only rarely extending posterior to ovary and never posterior to anterior testis ; seminal receptacle present or absent, usually absent ; eggs large ; vitelline follicles large ; genital pore preacetabular usually to the (left submarginal and dorsal in the Notoporinae). Adults in the intestine and caeca of fishes, usually marine fishes ; the cercariae known are of the cotylomicrocercous type, developing in sporocysts in snails ; Type genus *Opecoelus* Ozaki, 1925."

We accept the above diagnosis of the family and think that this family has arisen from the common ancestor of the Homalometridae, Allocreadiidae and Lepocreadiidae evolved as a branch from the Acanthocolpid ancestor. This common ancestor had spinulate body. The adults of the Allocreadiidae which have also smooth, more or less elongated and usually flattened body are sometimes difficult to distinguish from the Opecoelidae. The Allocreadiidae are, however, common in fresh water fishes while the Opecoelidae and Lepocreadiidae are very common in marine fishes.

Macroderoidae McMullen, 1937.

The family Macroderoidae McMullen, 1937 is maintained by us on account of the uterus descending to posterior end of body and then ascending, passing between two testes or dorsally and ventrally to them ; eggs small ; vitellaria lateral, of restricted size and extent, never extending mesially behind testes so as to unite in post-testicular region, but on the other hand usually ending in front of the level of testes or posterior testis *i.e.*, some distance in front of hinder end ; excretory vesicle tubular, I-shaped or saccular or Y-shaped with short arms extending to posterior or anterior testis. The cercaria is a xiphidiocercaria (*Macroderoides typicus*) having tail equal to one third or a little longer than body with a well defined finfold extending one-half to two-third of its length, a weak stylet and five pairs of stylet glands, four grouped in second quarter of body and fifth more anterior and difficult to see, discharging their secretion close to the base of the stylet. The excretory vesicle of xiphidiocercaria is I-shaped with a small dilatation at anterior end for the attachment of collecting tubules ; mesostomate protonephridia arising at a level with posterior border of acetabulum *i.e.*, where common collecting tubule divides into anterior and posterior tubules. The cercariae without eyes 1-4 in number develop in small rounded daughter sporocysts in gastropods (snails) and have spinulate body, fine spines being numerous at anterior end and sparse at posterior end. They penetrate and develop into metacercariae in tadpoles. Adults parasitic in intestine of fresh water and marine fishes.

Type genus : *Macroderoides* Pearse, 1924 syn. *Plesiocreadium* Winfield, 1929.

The family Macroderoidae McMullen, 1937 is derived from the Allocreadiidae having spinose or aspinose body. The testes are tandem, oblique or symmetrical. The cirrus sac containing vesicula seminalis, prostate complex and cirrus is present ; it does not extend much behind acetabulum. Vesicula seminalis externa is absent. Genital pore is median or submedian in front of acetabulum. The ovary is postacetabular or may overlap acetabulum and pretesticular. The restricted extent of the vitellaria and uterus extending to hinder end and then winding forwards in its course are characteristic features of the family, which distinguish it from the Allocreadiidae. The family Opecoelidae Ozaki is

also closely related to the latter family having been evolved from the immediate common ancestor but it is more advanced and specialised in certain features such as in cercariae being of cotylomicrocercous type developing in sausage-shaped sporocyst with terminal birth pore in marine, exceptionally in fresh water snails. Cercariae possess complex double pointed stylet, aspinose cuticle, three pairs of cephalic glands with a single lateral and two median ducts on each side, oval postacetabular excretory vesicle and mesostomate protonephridia. The metacercariae encyst in marine Amphipods and are progenatic. The adults usually possess anal openings.

Yamaguti (1958) has recently accepted it as a subfamily under the Allocreadiidae, but we accept it as a separate family as already shown, on account of the specific characters of the cercariae and the life cycle. It has been more and more realised that each family has a characteristic larval type. We, therefore, agree with Hunninen and Cable (1941), Hopkins (1941) and Manter (1947) that the Opecoelidae should be considered and recognised as a family.

Lepocreadiidae Nicoll, 1934.

Family diagnosis.—Allocreadioidea : Body small to medium sized rarely large, elongated, flattened, or oval to rounded, pyriform or foliate, spinulate rarely unspined. Remnants of cercarial eye spots usually present. Prepharynx varying in length, long, moderately long or unusually long or sometimes short or even absent. Pharynx present. Oesophagus usually short, sometimes moderately long, long or very short ; intestinal caeca always long ending blindly near hinder end or occasionally opening into excretory vesicle. Acetabulum usually small, sometime larger than oral sucker, rarely large ; at about anterior third or quarter of body length, sometimes equatorial or just pre-equatorial. Genital opening median, submedian, anterosinistral to left of oesophagus or intestinal bifurcation, median betwean the latter or pharynx and acetabulum, immediately postbifurcal or immediately preacetabular, median immediately behind acetabulum (Postporinae Yamaguti, 1958) or marginal or submarginal pretesticular between acetabulum and ovary (Opisthogonoporinae Yamaguti, 1937). Testes in posterior half of body, tandem, oblique, or sometime symmetrical near hinder end sometimes multiple *i.e.*, split up into follicles (*Rhagorchis* Manter, 1931 and *Multitestis* Manter, 1931, Folliorchiinae Yamaguti), rarely single (*Hairana* Nagaty, 1948, *Spiritestis* Nagaty, 1948). Cirrus sac as a rule well developed, rarely absent, sometime slender or weakly developed, usually elongated, claviform, extending behind acetabulum, sometimes even further behind between the latter and ovary, or preacetabular, enclosing tubular or saccular vesicula seminalis interna, prostatic complex and protrusible cirrus, and almost always accompanied by vesicula seminalis externa ; prostate gland cells partly outside and lateral to cirrus sac sometime present. Ovary usually pretesticular, postacetabular, occasionally intertesticular or opposite anterior testis. Receptaculum seminis present exceptionally absent. Laurer's canal present. Vitellaria extending along caeca from level of acetabulum or a little behind it to the hinder end, sometimes more extensively developed from level of pharynx or oesophagus or intestinal bifurcation ; follicles usually small, occasionally large. Uterus preovarian coiled between ovary and acetabulum, intercaecal, confined to region anterior to ovary or testes, occasionally winding into space between two testes. Eggs usually large, with one exception always without polar filaments. Excretory vesicle tubular, often long reaching acetabulum or even further forwards beyond intestinal bifurcation, occasionally short ending some distance behind acetabulum reaching to region of testes, exceptionally Y-shaped. Cercariae setiferous tailed bearing lateral tufts of setae on the long

tail, possessing conspicuous eye spots and lacking stylet, develop in rediae in gastropods. Metacercariae encysted or unencysted in marine invertebrates. parasitic in digestive tract of marine fishes, rarely in stomach or gut of fres fishes.

Type genus : *Lepocreadium* Stossich, 1904 syn. *Leptotrema* Ozaki, 1932.

The family Lepocreadiidae Nicoll as concieved by us is quite large. Though the life cycle of only a few genera is known, it is expected that it will be essentially similar to that of *Lepocreadium*, *Opechona* and *Stegodexamine* and that the occulate setiferous tailed cercaria is characteristic of the family. We consider it to be a compact group as defined above. Besides the setiferous tailed cercariae the other distinguishing features are the spinulate body-wall of the adult in contrast to the unspined body wall of the Allocreadiidae and Opecoelidae Ozaki, 1925, the presence of cirrus sac accompanied by vesicula seminalis externa ; the cirrus sac is rudimentry or absent in the Opecoelidae and the vesicula seminalis externa is absent in the Allocreadiidae. The excretory vesicle is long and tubular in the Lepocreadiidae, whereas it is short not extending as a rule beyond testes in the Allocreadiidae. The Allocreadiidae are mostly parasitic in gut of fresh water fishes, the Lepocreadiidae mostly in the digestive tract of marine fishes, exceptionally in fresh water fishes and the Opecoelidae in the intestine of usually marine fishes. The cerceariae of the latter family are of the cotylomicrocercous type, developing in sporocysts in snails. So the three families are quite distinct and well defined. Of these families, Lepocreadiidae is the most primitive and Opecoelidae the most advanced and specialised. The Allocreadiidae is closely related to the Homalometridae n. fam. and has probably arisen with it from the common Acanthocolpid ancestor evolving out fo the Echinostomoidea, from which the Lepocreadiidae arose as a side branch. The Deropristiidae n. fam. as has been already discussed also arose from the ancestor close to the Homalometridae.

In the family Lepocreadiidae are included the subfamilies Lepocreadiinae Odhner, 1905, Aephnidiogenetinae Yamaguti, 1934, Hypocreadiinae n. subf. created for *Hypocreadium* Ozaki, 1936 and *Pseudocreadium* Layman, 1930, Rhagorhiinae n. subf. for *Rhagorchis* Manter, 1931 and *Multitestis* Manter, 1931, Hairainae n. subf. for *Hairana* Nagaty, 1943, Stegodexamininae n. subf. for *Stegodexamene* Macfarlane, 1951, Eocreadinae n. subf. for *Eocreadium* Szidat, 1954 and Labriferinae Yamaguti, 1958. The subfamilies Trigonotrematinae Yamaguti, 1958, Spiritestinae Yamaguti, 1958, Opisthogonoporinae Yamaguti, 1958, Petalocotylinae Ozaki, 1937, Postporinae Yamaguti, 1958, and Orientocreadiinae Yamaguti, 1958 are also assigned to the family Lepocreadiidae. The subfamily Labriferinae Yamaguti, 1958 is dropped and held as a synonym of Petalocotylinae Ozaki, 1937. The family Dermadenidae Yamaguti is reduced to subfamily Dermadeninae n. subf. under this family.

Key to the subfamilies of Lepocrediidae

1. Body truncated posteriorly with lateral edges expanded to form wings or sleeves.....Trigonotrematinae Yamaguti, 1958.
   Body not truncated posteriorly and lateral edges not expanded to form wings or sleeves 2
2. Genital opening behind acetabulum 3
   Genital opening in front of acetabulum 4
   Genital opening to left at level of middle of acetabulum between it and body wall.....Folliorchiinae Yamaguti, 1958.

3. Genital opening marginal or submarginal, pretesticular between acetabulum and ovary ; cirrus sac present.....Opisthogonoporinae Yamaguti, 1958.

Genital opening median immediately behind acetabulum ; cirrus sac absent.....Postporinae Yamaguti, 1958.

4. Oral sucker with 4 or 8 anterior retractile musuclar processes ; ceaca short almost half as long as body.. Spiritestiinae Yamaguti, 1958.

Oral sucker without anterior muscular processes ... 5

5. Testis one.....Hairaninae n. subf.

Teatis multiple, split up into follicles.....Rhagorchiinae n. subf.

Testes two 6

6. Caeca arcuate ; testes almost parallel 7

Caeca non-arcuate ; testes tandem near hinder end, exceptionally parallel and immediately postequatorial 8

7. Ventral body surface with conspicuous dermal glands and papillae .....Dermadeninae n. subf.

Ventral dermal glands and papillae absent.....Hypocreadiinae n. subf.

8. Uterus extending to posterior extremity.....Orientocreadiinae Yamaguti, 1958.

Uterus anterior to ovary or testes 9

9. Acetabulum with parallel projections or lamellar lips..... Petalocotylinae Ozaki, 1937 syn. Labriferinae Yamaguti, 1958.

Acetabulum without projections or lamellar lips ... 10

10. Parasitic in fresh water fishes 11

Parasitic in marine fishes 12

11. Testes parallel, immediately postequatorial ; uterus between acetabulum and testes..... Eocreadiinae n. subf.

Testes oblique, almost equatorial ; uterus preovarian .....Stegodexamininae n. subf.

12. Genital opening median or submedian immediately preacetabular or between pharynx and acetabulum.....Acphnidiogenetinae Yamaguti, 1934.

Genital opening anterolateral or marginal, sinistral to acetabulum or marginal near oral sucker.....Lepocreadiinae Odhner, 1905.

Lepocreadiinae Odhner, 1905

Subfamily diagnosis.—Lepocreadiidae: Body elongated, pyriform, oval or plump, spinulate, usually oculate. Acetabulum pre-equatorial. Prepharynx distinct, moderately long, long or unusually long, sometimes short. Oesophagus short ; caeca reaching posterior end, occasionally opening into excretory vesicle. Testes tandem or oblique in posterior half of body. Cirrus sac clavate, extending usually posterior to acetabulum. Vesicula seminalis externa present ; prostate gland cells sometimes surrounding outside cirrus sac. Genital opening anterosinistral to acetabulum or anterolateral on left margin just in front of acetabulum or marginal near oral sucker. Ovary submedian or median, pretesticular, rarely

opposite anterior testis. Receptaculum seminis and Laurer's canal present. Uterus winding between ovary, anterior testis or posterior testis and acetabulum. Vitellaria lateral, extending from acetabulum or intestinal bifurcation to hinder end, usually united in postacetabular region. Excretory vesicle tubular, long, extending to acetabulum or beyond it. Parasitic in intestine of marine fishes.

Type genus : *Lepocreadium* Stossich, 1904 syn. *Lepotrema* Ozaki, 1932.

The other genera included are *Opechona* Looss, 1907 syn. *Pharyngora* Lebour, 1908, *Opechonoides* Yamaguti, 1940, *Lepocreadioides* Yamaguti, 1936, *Lepidepedon* Stafford, 1904 syn. *Lepodora* Odhner, 1905 and *Allolepedapedon* Yamaguti, 1940. We have dropped the subfamily Lepidapedinae Yamaguti, 1958 and have included its latter two genera in the Lepocreadiinae.

Aephnidiogenetinė Yamaguti, 1934

Subfamily diagnosis.—Lepocreadiidae : Body elongated, spinulate. Prepharynx short or moderately long or absent. Oesophagus short or very short; caeca reaching posterior end. Acetabulum small, in anterior third or fourth of body. Testes tandem, equatorial or postequatorial. Cirrus sac small, anterodorsal to acetabulum or not extending behind the latter. Vesicula seminalis externa winding and sometimes surrounded by prostate gland cells lying freely in parenchyma situated immediately behind acetabulum. Genital pore median or submedian immediately in front of acetabulum or between the latter and pharynx. Ovary entire or lobed immediately postacetabular. Vitellaria postacetabular extending from level of ovary or a little more forwards from acetabulum or behind level of vesicula seminalis externa to hinder end, united behind testes. Uterus winding between ovary or anterior testis and acetabulum. Excretory vesicle tubular extending to ovary or testicular zone or posterior testis. Parasitic in intestine of marine fishes.

Type genus : *Aephnidiogenes* Nicoll, 1915.

The other genera included are *Neolepidapedon* Manter, 1954, *Holorchis* Stossich, 1901 and *Pseudoholorchis* Yamaguti, 1958.

Eocreadiinae n. Subf.

Subfamily diagnosis.—Lepocreadiidae : Body spatulate with rounded extremities, unspinulate. Acetabulum pre-equatorial, smaller than oral sucker. Prepharynx very short ; oesophagus short ; intestinal caeca reaching near hinder end. Testes parallel, closely opposite to each other, immediately postequatorial. Cirrus sac narrow, slightly developed, elongated, mostly dorsal to acetabulum. Vesicula seminalis externa present. Genital pore median immediately in front of acetabulum. Ovary in front of right testis. Receptaculum seminis large. Vitellaria lateral, extending from level of acetabulum to posterior end and turning round caeca to surround them behind testes on each side of median tubular excretory vesicle. Uterus coiled, intercaecal between testes and acetabulum. Eggs large, not numerous. Parasitic in stomach of fresh water fishes.

Type genus : *Eocreadium* Szidat, 1954.

Stegodexamininae n. subf.

Subfamily diagnosis.—Lepocreadiidae : Body small, elongated, cylindrical, spinulate. Rudiments of larval eye spots present. Cuticular glands well developed. Suckers small, almost equal ; acetabulum pre-equatorial. Prepharynx very short ; oesophagus narrow and long ; caeca reaching posterior end. Testes

oblique, round, almost equatorial. Cirrus sac claviform, extending much behind acetabulum, containing bipartite vesicula seminalis, prostatic complex and muscular cirrus. Vesicula seminalis externa absent. Genital pore immediately preacetabular slightly to left of median line. Ovary dextral in front of posterior testis and with the two testes forming three angles of a triangle. Receptaculum seminis present. Vitellaria postacetabular, extending from level of ovary to hinder end, lateral around caeca and separate throughout their length. Uterus preovarian, intercaecal between ovary and acetabulum. Muscular metraterm present; eggs fairly large. Excretory vesicle long, tubular reaching intestinal bifurcation. Parasitic in digestive tract of fresh water fishes. Cercaria oculate with setiferous tail and four pairs of penetration glands.

Type genus : *Stegodexamine* Macfarlane, 1951.

Petalocotylinae Ozaki, 1937
syn. Labriferinae Yamaguti, 1958

Subfamily diagnosis.—Lepocreadiidae : Body elongated, unspinulate or spinulate. Prepharynx short or long or absent. Oesophagus short or absent; caeca terminating at or near hinder end. Acetabulum large, pre-equatorial, with petaloid marginal projections or muscular lamellar lips or semicircular bands of lamellar muscles. Testes tandem, in hinder half of body. Cirrus sac long, preacetabular or overlapping acetabulum extending to just behind acetabulum. Vesicula seminalis outside cirrus sac, free in parenchyma; pars prostatica surrounded by mass of prostate cells differentiated outside cirrus sac, postacetabular. Genital pore median or submedian to left side of oesophagus or intestinal bifurcation or immediately behind intestinal bifurcation. Ovary immediately pretesticular or between anterior testis and vesicula seminalis. Receptaculum seminis prasent. Vitellaria lateral around caeca, extending from in front of or from behind acetabulum to hinder end united in post-testicular region. Uterus coiled, preovarian. Excretory vesicle tubular, extending to ovary or testicular zone. Parasitic in intestine of marine fishes.

Type genus : *Petalocotyle* Ozaki, 1934.

The other genera included are *Labrifer* Yamaguti, 1936 and *Myzoxenus* Manter, 1934.

Orientocreadiinae Yamaguti, 1958

Subfamily diagnosis.—Lepocreadiidae : Body small elongate, spinulate. Prepharynx short or long. Oesophagus short or long; intestinal caeca terminating at hinder end. Acetabulum small, pre-equatorial, rarely equatorial. Testes tandem or oblique, postequatorial. Vesicula seminalis externa present. Cirrus sac muscular, claviform, to one side of acetabulum, enclosing vesicula seminalis, prostate complex and cirrus, not extending or slightly extending behind acetabulum. Genital pore median, immediately in front of acetabulum. Ovary submedian or median, postacetabular, closely in front of anterior testis. Receptaculum seminis absent. Uterus extending to hinder end, overreaching caeca laterally. Metraterm well developed. Vitellaria lateral, extending from behind or just in front of acetabulum to hinder end, not joining behind testes. Eggs small, numerous. Excretory vesicle tubular or Y-shaped with short arms. Parasitic in intestine of fresh water fishes.

Type genus : *Orientocreadium* Tubangui, 1931 syn. *Ganada* Chatterji, 1932, *Nizamia* Dayal, 1938, *Ganadotrema* Dayal, 1949 and *Pratamopsolus* Dubinina et Bychovsky, 1954.

The other genus included is *Macrotrema* Gupta, 1951.

Hypocreadiinae n. subf.

Subfamily diagnosis.—Lepocreadiidae : Body round, oval, pyriform or elliptical, spinulate. Acetabulum small, equatorial or just pre-equatorial. Prepharynx short ; intestinal caeca arcuate, terminating near or at hinder end. Testes parallel or diagonal near hinder end. Cirrus sac claviform preacetabular or mostly in front of acetabulum with its posterior end overlapping the latter. Vesicula seminalis externa present. Genital pore to left of intestinal bifurcation, oesophagus or pharynx. Ovary submedian or median, between two testes or just pretesticular. Receptaculum seminis and Laurer's canal present. Uterus between two testes, sometime extending close to posterior end or between testes and acetabulum. Vitellaria extensively and profusely developed around caeca from pharynx or oral sucker to hinder end and united mesially behind testes. Excretory vesicle tubular extending to acetabulum or further forward ; excretory pore terminal or dorsal. Parasitic in digestive tract of marine fishes.

Type genus : *Hypocreadium* Ozaki, 1936.

The other genus included is *Pseudocreadium* Layman, 1930 syn. *Leptocreadium* Ozaki, 1936.

Hairaninae n. subf.

Subfamily diagnosis.—Lepocreadiidae : Body elongated, spinulate. Acetabulum near anterior end. Prepharynx absent ; oesophagus short ; caeca long reaching hinder end. Testis single, postequatorial near posterior end. Cirrus sac well developed, containing vesicula seminalis interna. Vesicula seminalis externa present. Genital pore median in front of acetabulum. Ovary median, pretesticular. Receptaculum seminis absent. Uterus preovarian, opening into metraterm pouch. Vitellaria extensively developed from acetabulum to hinder end, united mesially behind testes and between it and ovary. Eggs numerous. Parasitic in marine fishes.

Type genus : *Hairana* Nagaty, 1948.

Rhagorchiinae n. subf.

Subfamily diagnosis.—Lepocreadiidae : Body elongated, slightly elongated or oval, somewhat fusiform or plump, spined anteriorly. Acetabulum in anterior half of body. Prepharynx absent ; oesophagus short or very short ; caeca terminating near or at hinder end. Testes multiple, *i. e.*, split up into follicles 11 in one median group with ovary in front or 10 or 11 in two median or lateral groups with ovary between them. Cirrus sac elongated, claviform, extending behind acetabulum. Vesicula seminalis externa present. Genital pore to left of oesophagus or anterolateral to acetabulum. Ovary pretesticular or intertesticular, lobed, median or submedian, equatorial or just pre-equatorial. Receptaculum seminis present. Uterus preovarian or may extend posterior to ovary. Vitellaria lateral, extending from level of ovary or level of oesophagus or intestinal bifurcation to hinder end. Excretory vesicle extending to behind acetabulum. Parasitic in intestine of marine fishes.

Type genus : *Rhagorchis* Manter, 1931 syn. *Gargorchis* Linton, 1940.

The other genus included is *Multitestis* Manter, 1931.

Postporinae Yamaguti, 1958

Subfamily diagnosis.—Lepocreadiidae : Body elongated, spinulate, oculate. Acetabulum pre-equatorial, smaller than oral sucker. Prepharynx absent ; pharynx large ; oesophagus short ; caeca terminating near hinder end. Testes tandem near hinder end. Cirrus sac absent. Vesicula seminalis coiled free in parenchyma. Tubular pars prostatica present but prostate cells absent. Genital pore median, immediately behind acetabulum. Ovary pretesticular. Receptaculum seminis and Laurer's canal present. Uterus coiled, preovarian. Vitellaria lateral extending from level of acetabulum to hinder end, united behind testes. Excretory vesicle tubular, long, reaching intestinal bifurcation or pharynx. Parasitic in intestine of marine fishes.

Type genus : *Postporus* Manter, 1949 syn. *Opisthoporus* Manter, 1947.

Opisthogonoporinae Yamaguti, 1958

Subfamily diagnosis.—Lepocreadiidae : Body elongated, spinulate. Acetabulum in anterior third of body. Prepharynx present. Pharynx large ; oesophagus moderately long ; caeca terminating near hinder end. Testes tandem, close in front of each other in posterior half of body. Cirrus sac transversely situated between acetabulum and ovary enclosing bipartite vesicula seminalis and protrusible cirrus. Vesicula seminalis externa present. Genital opening submarginal or marginal, postacetabular almost midway between ovary and acetabulum. Ovary median, closely in front of anterior testis. Receptaculum seminis, Laurer's canal and shell gland mass anterior to ovary. Uterus with a few coils, enlarged terminally into egg reservoir. Vitellaria lateral around caeca, extending from level of ovary to hinder end. Excratory vesicle tubular, short, reaching posterior testis. Parasitic in intestine of marine fishes.

Type genus : *Opisthogonoporus* Yamaguti, 1937.

Folliorchiinae Yamaguti, 1958

Subfamily diagnosis.—Lepocreadiidae : Body ovoid, unspinulate. Acetabulum slightly larger than oral sucker, equatorial. Prepharynx absent. Oesophagus absent ; intestinal caeca reaching posterior end. Testes follicular, intercaecal, aggregated as a large number of small follicles at hinder end of body. Cirrus sac enclosing vesicula seminalis interna, prostatic complex and ductus ejaculatorius. Genital pore lateral situated at level of middle of acetabulum between it and left body wall. Vesicula seminalis externa present extending posteriorly reaching anterior testis. Ovary spherical, pretesticular. Uterus confined to space between acetabulum and testes. Vitellaria composed of small follicles extending along caeca from level of intestinal bifurcation to hinder end, contiguous behind. Parasitic in intestine of marine food fishes.

Type genus : *Felliorchis* Srivastava, 1948.

The subfamily Orientiocreadiinae Yamaguti included by us in the Lepocreadiidae stands intermediate between the latter family and the family Macroderoidae McMullen, 1937, which it resembles in the extent of its uterus, extending to the posterior end of the body and then ascending between the testes. It also resembles in the vitellaria being of restricted extent and not united behind the testes. Tubangui (1931) pointed out that his new genus *Orientocreadium* "bears

certain resemblance to *Plesiocreadium typicum* Winfield, 1929, but it differs from the latter in the position of the seminal vesicle outside the cirrus sac, presence of a prepharynx, shortness of the oesophagus, and in the posterior extent of the vitelline glands." We drop the subfamlly Walliniinae Yamaguti, 1958. The family Macroderoidae McMullen, 1937 is also distinguished as has been already mentioned in possessing characteristic xiphidiocercaria having tail about equal to one third or a little longer than the body with a well defined finfold extending over one half to two third of its length, a weak stylet and five pairs of stylet glands. It is likely that the Macroderoidae McMullen, 1937 has been evolved from the Lepocreadiidae, which is a primitive family of the Allocreadioidea through the subfamily Orientocreadiinae.

Trigonotrematinae Yamaguti, 1958

Subfamily diagnosis.—Lepocreadiidae : Body small, truncated behind with lateral edges expanded and turned ventrally in the form of symmetrical wings or sleeves. Acetabulum small, pre-equatorial. Prepharynx short. Oesophagus very short ; caeca long reaching hinder end. Testes immediately behind acetabulum, symmetrically opposite to one another, mesially to caeca. Cirrus sac clavate, preacetabular, enclosing vesicula seminalis interna, prostate complex and cirrus. Vesicula seminalis externa present. Genital opening on left body margin in level with pharynx. Ovary submedian, immediately behind acetabulum, intertesticular. Receptaculum seminis and Laurer's canal present. Uterus short, preovarian ; metraterm well differentiated. Vitellaria occupying post-testicular area mesially and in the region of wings. Excretory vesicle strongly curved in the form of an interrogation mark reaching close behind intestinal bifurcation ; excretory pore terminal. Parasitic in intestine of marine fishes.

Type genus : *Trigonotrema* Goto and Ozaki, 1929.

Spiritestiinae Yamaguti, 1958

Subfamily diagnosis.—Lepocreadiidae : Body elongated, spinulate. Oral sucker large with 4 or 8 retractile muscular processes anteriorly. Acetabulum smaller than oral sucker, pre-equatorial. Prepharynx almost absent. Oesophagus short ; caeca terminating much in front of hinder end, almost half the length of body. Testis single, median, elongated, more or less spiral in posterior half of body. Cirrus sac elongated, lateral to acetabulum. Vesicula seminalis externa present. Genital opening preacetabular. Ovary pretesticular with shell gland complex in front. Uterus preovarian ; metraterm pouch shaped. Receptaculum seminis uterinum present. Eggs a few and large. Parasites of marine fishes.

Type genus : *Spiritestis* Nagaty, 1948.

Dermadeninae n. subf.

Subfamily diagnosis.—Lepocreadiidae : Body circular or semicircular, spinate with evanescent subcircular cuticular scales. Ventral body surface provided with numerous conspicuous multicellular glands and papillae with pore at end ; oculate. Suckers well developed ; acetabulum slightly larger than oral sucker, subequatorial. Prepharynx absent ; pharynx well developed ; oesophagus short ; caeca arcuate, narrow, quite apart from body edges nearer median line, curving round gonads, ends not far apart terminating near posterior end. Genital pore submedian, slightly to left of pharynx, prebifurcal. Testes two, symmetrical, intercaecal separated by ovary, immediately postacetabular. Cirrus sac elongated clavate extending diagonally backwards to slightly behind right anterior edge of

acetabulum. Vesicula seminalis externa present; cirrus large, armed with tubercles or papillae. Ovary median intertesticular. Receptaculum seminis large, anterior to ovary. Vitellaria widely distributed on dorsal surface from level of intestinal bifurcation to near posterior end filling wide extracaecal fields. Uterus short, coiled, preovarian immediately postacetabular. Metraterm well developed. Eggs a few. Excretory vesicle Y-shaped with arms probably representing enlarged collecting tubules. Parasitic in marine fishes.

Type genus : *Dermadena* Manter, 1945.

The family Dermadenidae Yamaguti, 1958 for the genus *Dermadena* Manter is dropped. Manter rightly mentions that the latter genus resembles *Pseudocreadium* Layman, 1930. There is no doubt that this genus stands close to *Pseudocreadium* Layman, 1930, *Eurycreadium* Manter, 1934, *Lepocreadium* Stossich, 1904 and *Hypocreadium* Ozaki, 1936. Though Manter (1940) considers *Hypocreadium* Ozaki, 1936 a synonym of *Pseudocreadium*, he (1945), however mentions the latter can be distinguished on account of the preovarian receptaculum seminis and intertesticular ovary in *Hypocreadium*. We agree in this view and maintains it as a separate genus. Yamaguti (1958) has also done this.

The genus *Dermadena* Manter resembles most closely the genus *Hypocreadium* and *Pseudocreadium*. Its characteristic feature is development of conspicuous multicellular glands and papillae on ventral body wall. The presence of ventral glands of *Dermadena* resemble according to Manter the papilla like and retractile glands of the Notocotylidae. On comparing it with the latter family he points out that the latter lacks an acetabulum, a pharynx and seminal receptacle. There are, however, some remarkable features of similarity such as symmetrical testes, intertesticular ovary, dorsal excretory pore and Y-shapped excretory bladder, and presence of vesicula seminalis externa. But he rightly concludes to place it in the Lepocreadiidae ; its relationship with the Notocotylidae is quite remote.

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THE EFFECT OF TEMPERATURE ON THE ACTIVITY OF HONEY BEE HEAD CHOLINESTERASE

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The honey bee, *Apis mellifera gandhiana* Nougiera Netto. is a benificial insect of great economic importance as it provides honey and serves as a pollinator to most of our commercial fruits as well as legume crops. Due to large scale use of more potent insecticides they are exposed to hazardous chemicals present in the environment. The temperature is an important factor which determines action of insecticides on the enzymes present in the system of bees.

For this experiment worker honey bees were collected and anaesthetised in di-ethyl ether and immediately frozen by keeping inside frigidaire. Honey bee heads were then seperated and kept in a beaker containing buffer solution (Nacl, 26·30 gm., KH_2PO_4, 3·85 gm., NaOH, 1·00 gm., H_2O to make 1 litre pH adjusted to 8·0). These heads were ground and filtered and the volume of the buffer solution adjusted so as to contain one bee head per c.c. Inactivation of the enzyme was done by incubating suspensions at the chosen temperatures for various periods of time.

Assessment of the rate of inactivation was made at half-hourly intervals by measuring the change in p-H by p-H meter after adding acetylcholine bromide (Ach Br) 0·015 M as substrate. In this way the effect of temperature was measured at 20°, 30°, 35° and 40° Centigrade. The results are shown in the table I.

RESULT

The temperature dependence of the cholinesterase activity in the ground heads of the honey bee has been found to be maximum at 35°C.

TABLE I

The effect of temperature on the Cholinesterase activity of Honey bee Head

Temperature	Quantity of bee's brei in buffer solution and substrate.	Initial p-H	1st hour		2nd hour		3rd hour		4th hour		Remarks
			30 Minutes after	30 Minutes after	30 Minutes after	30 Minutes after	30 Minutes after	30 Minutes after	30 Minutes after	30 Minutes after	
20°C	A. 10 c.c. of bee's head brei in buffer solution + 10 c.c. of substrate	8·00	7·5	7·3	7·2	7·15	7·05	7·05	7·00	7·00	
	B. do	8·00	7·5	7·35	7·2	7·15	7·05	7·05	7·00	7·00	
	C. 10 c.c. of buffer sol. + 10 c.c. of substrate	8·00	7·5	7·5	7·5	7·5	7·5	7·5	7·5	7·5	
30°C	A. 10 c.c. of bee's head brei in buffer sol. + 10 c.c. of substrate	8·00	7·5	7·3	7·2	7·05	7·00	7·00	6·9	6·9	
	B. do	8·00	7·5	7·3	7·2	7·05	7·00	7·00	6·9	6·9	
	C. 10 c.c. of buffer sol. + 10 c.c. of substrate	8·00	7·5	7·5	7·5	7·5	7·5	7·5	7·5	7·5	
35°C	A. 10 c.c. of bee's head brei in buffer sol. + 10 c.c. of substrate	8·00	7·5	7·3	7·15	7·1	7·00	6·9	6·85	6·85	
	B. do	8·00	7·5	7·3	7·15	7·1	7·00	6·9	7·85	6·85	
	C. 10 c.c. of buffer sol. + 10 c.c. of substrate	8·00	7·5	7·5	7·5	7·5	7·5	7·5	7·5	7·5	
40°C	A. 10 c.c. of bee's head brei in buffer sol. + 10 c.c. of substrate	8·00	7·5	7·3	7·05	7·00	6·95	5·95	6·95	6·95	
	B. do	8·00	7·5	7·5	7·05	7·00	6·95	6·95	6·95	6·95	
	C. 10 c.c. of buffer sol. + 10 c.c. of substrate	8·00	7·5	7·5	7·5	7·5	7·5	7·5	7·5	7·5	

VEGETATION OF SUTGATTI, WESTERN GHATS

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The present paper deals with the vegetation of Sutgatti and adjoining areas in the district of Belgaum in Western Ghats. The area of study comprises of a small mountaneous tract with an average altitude ranging between 400–800 meters above sea-level. The low hillocks here, which rise upto an elevation of 800 meters, are generally covered with deciduous forests of Teak, *Terminalia, Anogeissus, Legerstroemia,* etc. The slopes of these hills gradually merge into valleys which are extensively put under cultivation of paddy and other cereals.

The principal drainage is provided by the Ghatprabha river and its tributaries, flowing eastwards into Bay of Bengal.

The climate of the area is typically monsoonic with an average annual rainfall of about 144 cms.

Soil and Geology:

Two distinct soil types are met with in the area *i.e.*, Black and Red soils, both being derived from basalt (trap). The black soils are of alluvial type and support a deciduous vegetation of Teak type while the red soils, generally, have a poor teak growth. The black soils are common along the slopes and valleys while on higher levels red soils predominate.

The data has been presented on the basis of study of quadrat (Misra and Puri, 1954). The size of the quadrat was determined by the species–area–curve method. The minimum size of the quadrat was found to be 6 m. × 6 m. for the forest and 3 m. × 3 m. for the scrub jungle (fig. 1).

Vegatation :

The deciduous forests of the area may be classified into two types *i.e.*, (1) Moist-deciduous and (2) Dry-deciduous forests.

Again with teak as the indicator species, vegetation types may be split into (1) *Teak forests* and (2) *Non-Teak forests.*

The climax stable type in the teak forests is a community *Teak-Terminalia* type while in Non-teak *Terminalia-Emblica* subsists. The other communities in the area are various stages in the development of these two climax communities. The scrub jungles are biotic or bioedaphic, representing a secondary vegetation which under protection will develop into a deciduous forest.

Teak Forests :

Besides the climax community *i.e., Teak-Terminalia,* four other communities have been studied and their analysis is presented as follows :.

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(i) *Teak-Terminalia community* :

The community has been studied in the areas of *Ukkad*, slope facing North East and *Sutgatti*, slope facing East. Table I gives the summary of data to show the percentage frequency of the various species in the community.

TABLE I

Teak-Terminalia community

Name of the forest	Sutgatti	Ukkad
Aspect	Slope facing east	Slop facing N. E.
Altitude (approx.)	500 meters	500 meters
Soil	Alluvium	Alluvium
No. of quadrats studied	8	8
Name of the plant	Percentage frequency of the species	
Trees :		
Anogeissus latifolia	50	37·5
Chloroxylon sweitenia	37·5	12·5
Emblica officinalis	–	25
Lagerstroemia parviflora	25	12·5
Saccopetalum tomentosum	–	37·5
Tectona grandis	100	100
Terminalia tomentosa	100	100
Shrubs and Climbers :		
Acacia concinna	25	60
Carissa congesta	–	25
Cryptolepis buchnani	–	75
Dioscorea bulbifera	75	75
Dioscorea oppositifolia	75	75
Gymnosporia sp.	37·5	–
Lantana camara	100	87·5
Meyna laxiflora	25	–
Randia dumetorum	100	75
Ground flora :		
Anogeissus latifolia	25	–
Asparagus racemosus	75	75
Biophytum sensitivum	100	50
Curculigo orchioides	100	75
Tectona grandis	50	50
Terminalia tomentosa	25	50

(ii) Teak-Anogeissus-Chloroxylon community :

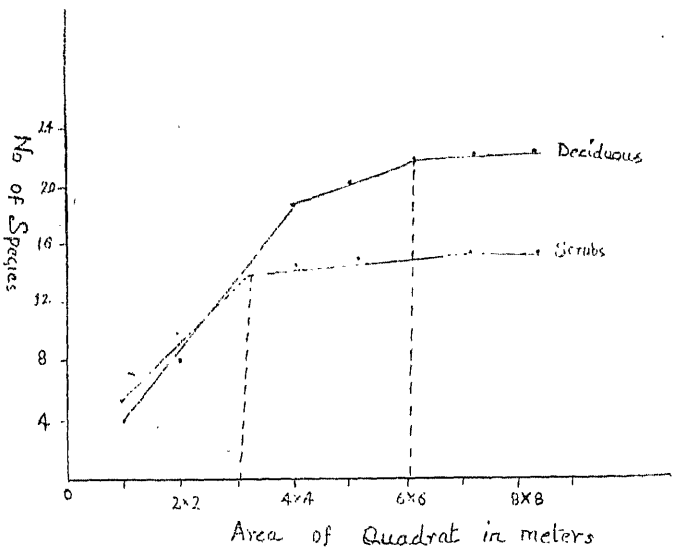
This community has been studied at Mangutti forest by taking 10 quadrats. The forest is open with trees not exceeding 8-9 meters in height. The percentage frequency of Teak is 100 while that of *Anogeissus latifolia* and *Chloroxylon sweitenia* is 90. The other common associates here are *Terminalia tomentosa, Legerstroemia parviflora, Diospyros melanoxylon* and *Cassia fistula.*

The dominant shrubs are *Lantana camara, Randia tetrasperma,* having percentage frequency of 100.

In the undergrowth, the regeneration of *Tectona grandis,* is common with few seedlings of *Anogeissus latifolia, Chloroxylon sweitenia* and *Terminalia tomentosa.* The common herbs are *Alysicarpus* sp., *Biophytum sensitivum, Curculigo orchioides, Eragrostis bifaria, Justicia simplex, Senecio tenuifolius* etc.

PLATE I

Fig. 1



(iii) Teak-Anogeissus-Grewia-Chloroxylon community :

This type has been studied at Sutgatti by taking 10 quadrats, along gentle slopes facing west. The forest is better developed than the above type. The percentage frequency of *Tectona grandis* here is 100 while that of *Anogeissus latifolia* is 70 and of *Grewia tiliaefolia* and *Chloroxylon sweitenia* is 40. Other common trees are *Buchnania latifolia, Cassia fistula, Diospyros melanoxylon, Erinocarpus nimonii, Santalum album* and *Lagerstroemia parviflora.*

The common shrubby components are, *Lantana camara*, and *Randia dumetorum*, with climbers like *Cryptolepis buchnani*, *Dioscorea bulbifera*, *D. oppositifolia*, *Vitis elongata* and *Zizyphus oenoplia*.

The common herbs are *Alysicarpus lineata*, *Biophytum sensitivum*, *Curculigo orchioides*, *Rungia parviflora*, and *Setaria glauca*.

(*iv*) *Chloroxylon-Teak-Lagerstroemia community* :

This community has been studied in Kattabali forest by taking 10 quadrats, along the slope facing west. The forest is open with more of shrubby associates. The percentage frequency of *Chloroxylon sweitenia* here is 100 while that of *Tectona grandis* and *Lagerstroemia* is 80. The other common trees are *Cassia fistula*, *Anogeissus latifolia*, *Diospyros melanoxylon* and *Terminalia tomentosa*.

Among the shrubs *Lantana camara*, *Randia dumetorum* are dominant while bushes of *Dodonea viscosa*, *Gymnosporia montana*, *Strychnos nux-vomica* are also present. The common climbers in the forest are *Dioscorea bulbifera*, *D. oppositifolia* and *Vitis gigantea*.

The ground flora is composed of the seedlings of dominant species and few herbs. The common herbs are *Clerodendron serratum*, *Chlorophyon* sp., *Eragrostis pilosa*, and *Senecio tenuifolius*.

(*v*) *Chloroxylon-Anogeissus-Teak community* :

This community has been studied in the scrub jungle having only three tree species that have very stunted growth. The percentage frequency of *Anogeissus latifolia* and *Chloroxylon sweitenia* in the 10 quadrats studied here is 100 while that of *Tectona grandis* is 60.

The dominant shrubs of the forest are *Lantana camara*, *Randia tetrasperma*, *Gymnosporia montana*, while the common shrubs are *Carissa congesta*, *Dodonea viscosa* and *Ixora parviflora*.

Among the herbs, *Alysicarpus* sp., *Eranthemum* sp. *Rungia parviflora*, *Spermacoce hispida*, *Tridax procumbens*, are common in the forest.

Non teak forest :

Among the Non-Teak areas the climax (stable) type is represented by *Terminalia-Emblica* community. Besides this two other communities of this type have been studied.

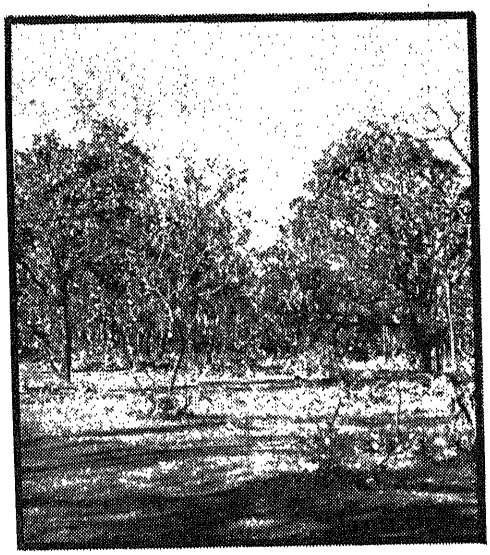
(*i*) *Terminalia-Emblica community* :

This community has been studied in the forest of Ukkad, situated on almost plane level with a gentle slope facing south west. The percentage frequency of *Terminalia tomentosa* here is 100 while that of *Emblica officinalis* is 80. The details of this community are given in table II.

PLATE II

Photo. 1. *Chloroxylon—Teak–Lagerstroemia* Community.

2.—*Teak—Terminalia* Community.



3. *Teak—Anogeissus-Chloroxylon* Community.

PLATE III

4. Trees of *Buchnania latifolia* in the background is the forest of kattabli.

5. *Terminalia–Phyllanthus* Community.

TABLE II

Terminalia-Emblica community.

Name of the forest	Ukkad
Aspect	S/W
Soil	Alluvium
No. of quadrats studied	15

	Name of the plant	Percentage frequency
Trees :		
	Buchnania latifolia	26·6
	Diospyros melanoxylon	13·3
	Emblica officinalis	80
	Garuga pinnata	13·3
	Plerocarpus marsupium	40
	Santalum album	26·6
	Terminalia tomentosa	100
Shrubs and Climbers :		
	Carissa congesta	13·3
	Cryptolepis buchnani	26·6
	Cyclea peltata	53·2
	Lantana camara	100
	Randia dumetorum	53·2
	Zizyphus xylopyrus	40
Ground flora :		
	Ageratum conyzoides	75
	Adiantum lunolarium	26·6
	Curculigo orchioides	100
	Dactylectenum aegypliacum	53·2
	Emblica officinalis	26·6
	Tectona grandis	26·6
	Thunbergia fragrans	70

(*ii*) *Lagerstroemia-Anogeissus-Diospyros community* :

This is an open scrub jungle having few tree species exceeding not more than 3 meters in height. 10 quadrats were studied. The percentage frequency of *Lagerstroemia parviflora*, is 100 while that of *Anogeissus latifolia* and *Diospyros melanoxylon* is 40 and 30 respectively. Bushes of *Phoenix* sp., are also common.

The dominant shrubs having percentage frequency of 100 are *Gymnosporia montana, Lantana camara, Randia tetrasperma, Dodonea viscosa.* Other common shrubs are *Opuntia* sp., and *Argyrea cuneata.*

The ground flora is composed of few small herbs here and there of which the common ones are *Alysicarpus* sp., *Apluda varia*, *Senecio tenuifolius* and *Tridax procumbens*.

(*iii*) *Chloroxylon-Terminalia community* :

The forest presents an appearance of the above type. The percentage frequency of *Chloroxylon sweitenia* and *Terminalia tomentosa* is 100 in the 10 quadrats studied. Other common trees are *Lagerstroemia parviflora* and *Anogeissus latifolia*.

The common shrubs are the hardy/thorny species of *Lantana camara*, and *Randia tetrasperma* (having percentage frequency of 100) and others *i.e.*, *Acacia* sp., *Dodonea viscosa*, *Fluegia microcarpus*, *Gymnosporia montana* and *Ixora parviflora*.

The common herbs are *Senecio tenuifolius*, *Rungia parviflora*, *Tridax procumbens*, *Setaria glauca* and others.

DISCUSSION

The stable forest in the area is *Teak-Terminalia* type in teak forests and *Terminalia-Emblica* type in non-teak forests. All other communities are developmental stages tending to progress towards the climax at different stages. The persistance of several communities in a more or less semi-permanent state is due to the *aspect*, *geology*, *nature of soil* and *biotic conditions*. The biotic factors have brought into existence a number of communities which are different from early stages and represent a secondary succession. Successional details in the area are being worked out.

I am grteaful to Dr. G. S. Puri, Director, Central Botanical Laboratory for his valuable guidance and to C. S. I. R. for the award of a Research Fellowship.

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ON THE MANNER OF THE DISCHARGE OF SECRETION FROM THE EPITHELIAL CELLS IN MIDGUT AND HEPATIC CAECA OF CERTAIN INSECTS*

By

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INTRODUCTION

It has been claimed by some workers that the secretion from midgut and hepatic caeca of insects may go on without any visible change in the structure of the cells. But most of the other workers have described a number of visible changes during the secretory activity of these cells and a large number of structures as discharged cytoplasmic globules, extruded nuclei etc. have been considered to represent secretory discharges. The present author therefore, decided to find out if there were some visible changes in the structure and activity of the epithelial cells in midgut and hepatic caeca of certain insects during normally fed conditions, unfed conditions and conditions at regular intervals after feeding.

MATERIALS AND METHODS

Three insects were employed for this study. These were : *Leogryllus bimaculatus* Sauss., *Periplaneta americana* Linn. and *Gryllodes sigillatus* Walk. Specimens of the three insects were collected and regularly fed for fifteen days. They were then dissected under following conditions :

(*a*) Some insects fed for fifteen days were dissected 6 hours after last feeding. These were regarded as normally feeding individuals.

(*b*) Some insects, which had been fed for fifteen days, were kept without food for 2 days after which they were dissected. These were regarded as unfed individuals.

(*c*) Some insects, which had been previously fed for fifteen days, were kept without food for two days. These were later fed and dissected at regular intervals of 3 hours, 6 hours and 9 hours after feeding. These were regarded as fed individuals.

The anterior portions of their midgut and their hepatic caeca were fixed in Mann-Kopsch and Yao-Nan fluids and 5 μ thick sections were obtained for the study.

OBSERVATIONS

The form and structure of the epithelial cells remain essentially similar in normally fed condition (Fig. 1), unfed condition (Fig. 2) and in conditions at regular intervals after feeding (Figs. 3 & 4). The columnar epithelial cells are at times of equal size but mostly they are arranged in groups of elongated and short cells. This change in the size of the cells shows no co-ordination with the secretory activity. The cytoplasm in the cells is granular in nature, the granules being

* Part of the thesis approved for the Ph.D. Degree of Lucknow University.

coarser in older elongated cells specially in the apical regions. The nuclei also present a uniformly similar structure under all conditions. The nuclear membrane is always intact and at no stage any nuclear extrusion has been observed. The nuclei generally occupy the middle region of the cells. The occasional shifting of the nuclei towards the apical regions of the cells takes place only in older cells in which the basal regions have become elongated. This occurs due to the age of the cells and increased pressure from the sides because of the growing younger cells formed by the regenerative cells. Thus the position of the nucleus also does not show any co-ordination to the secretory activity. The lumen ends of the columnar cells generally present an even surface without any trace of extruding protoplasmic globules, nuclei, etc. Cell extrusions often observed are too small in number to suggest any significance in the matter of secretory activity. If these cell extrusions represented secretory discharges as supposed by previous workers they should have shown a decrease in their number or even absence in the unfed individuals and their increase during normally fed condition or atleast in some other condition some time after feeding but such a thing does not occur at all. These cell extrusions may be observed in unfed individuals (Fig. 2) and do not show an increase in their number in normally fed condition (Fig. 1) or at any time after feeding (Figs. 3 & 4) and instead may be entirely absent in actively feeding individuals (Fig. 1). These facts afford conclusive evidence in support of the view that these cell extrusions cannot be regarded to be secretory discharges. Besides, these cell extrusions always occur on top of old cells and represent extrusion of old worn-out cells.

DISCUSSION AND CONCLUSIONS

There has been a good deal of confusion about the manner of discharge of secretion from the cells of midgut and hepatic caeca of insects. The earliest classical work concerning these is that by Gehuchten (1890) on *Ptychoptera contaminata*. He stated that the globular protrusions found on top of certain epithelial cells were secretory globules. Majority of later workers appears to have taken Gehuchten's (1890) conclusions for granted and have described the discharge of secretion from the epithelial cells in midgut and hepatic caeca of insects in form of these discharged cytoplasmic globules. Poyarkoff (1910) described discharge of secretory globules. Gresson (1934) described in case of *Periplaneta orientalis* separation of cell tips, discharge of cytoplasmic globules and bursting of cells as evidences of the secretory activity. Hodge (1936) working on *Melanoplus* had a similar view. Saksena (1951) working on the alimentary canal of *Aulacophora* also considered the discharge of secretion to take place in form of such structures as cytoplasmic globules, etc. Pradhan (1936) observed presence of such structures but did not mention whether he considered these to be secretory discharges or not in order to avoid a controversy (as stated by him in his paper). On the other hand, some workers believed that discharge of secretion takes place without the cell showing any visible change in appearance. Henson (1929, 1931) believed that the secretion goes on without any change in the epithelial cells in *Vanessa urticae*. Green (1931, 1933) stated that in case of *Vespa* secretion may be discharged without any alteration in the cells. Day and Powning (1949) working on the epithelial cells on *Blatella* clearly showed that the secretion is discharged by the cells in a simple manner of diffusion. Wigglesworth (1953) also holds a similar view in case of mosquito. The present author on the basis of his observations and reasons described above believes that the cell extrusions often observed along the epithelial surface are the results of cellular degeneration and do not represent secretory discharges and the secretion from the cells is discharged without any visible change in the epithelial cells in a simple manner of diffusion.

Fig. 1

Fig. 2

Fig. 3

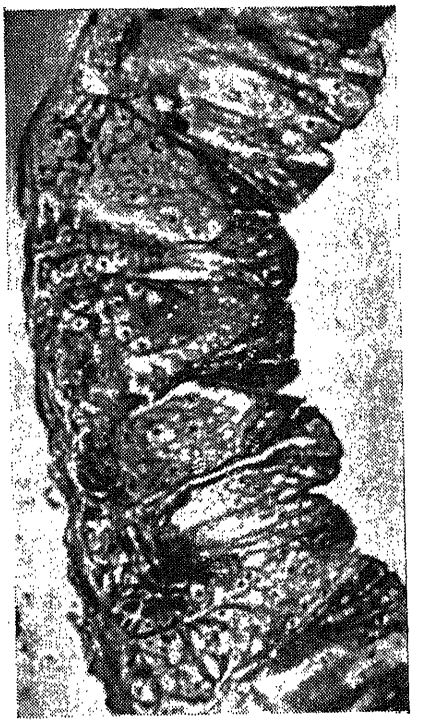

Fig. 4

EXPLANATION OF FIGURES (*Photomicrographs*)

Fig. 1. A part of epithelium from normally feeding specimen of *Leogryllus bimaculatus* Sauss. showing entire absence of cell extrusions. Yao-Nan preparation.

Fig. 2. A part of epithelium from unfed specimen of *Leogryllus bimaculatus* Sauss. showing presence of cell extrusions.

Fig. 3. A part of epithelium from specimen of *Leogryllus bimaculatus* Sauss. dissected 6 hours after feeding showing absence of cell extrusions. Yao-Nan preparation.

Fig. 4. A part of epithelium from specimen of *Leogryllus bimaculatus* Sauss. dissected 9 hours after feeding showing absence of cell extrusions. Yao-Nan preparation.

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SOME PHYSIOLOGICAL STUDIES ON SPHAEROPSIDALES

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INTRODUCTION

Growth, which involves an increase in both the number and mass of cells, is a complex process. In all the organisms, including fungi, growth follows a definite pattern and the way this development takes place depends upon the species, the environmental and the nutritional conditions. Temperature is one of the most important environmental factors for the growth of all the organisms. A large amount of literature has accumulated on the range of temperature suitable for growth of various fungi. Coons (1916) found that temperature limit of *Plenodomus fuscomaculans* was between 0°C and 30°C. The ranges of temperature for *Monilia fructigena* and *Cephalothecium roseum* (Ames, 1915) are 4°C--30°C and 9°C--35°C respectively while the lower temperature limit of *Diplodia natalensis* (Fawcett, 1921) was 50°F. These and many other reports confirm that different organisms differ in their growth rate at various temperatures.

Amongst nutritional requirements carbon is of fundamental importance for fungal growth. Almost half of the dry weight of the fungal cell consists of carbon. Generally the carbohydrates are preferred by fungi as the source of nutrition but no single sugar is the best source of carbon for all the fungi. However, most of the fungi can use a large variety of carbon sources for their growth and sporulation. It has been reported that species of the same genus differ in utilizing the same source of carbon. *Colletotrichum lini* (Tochinai, 1926) and *Gloeosporium psidii* (Agarwala, 1955) grew poorly on glycerol, but *Colletotrichum phomoides* (Kendrick and Walker, 1948) and *Gloeosporium musarum* (Grewal, 1954) utilized it favourably. Amongst Sphaeropsidales Kinsel (1937) and Stevens and Larch (1939) reported that *Diplodia macrospora* did not utilize the monosaccharides at all and grew only on the disaccharides. Bilgrami (1956) found that *Phyllosticta cycadina* and *Phyllosticta artocarpina* could utilize carbon from all the sources used by him, *viz.*, carbohydrates, sugar alcohols and organic acids.

For sulphur requirements Fruton and Simmonds (1958, p. 799) reported that "The nutritional requirements of various types of micro-organisms for sulphur vary from the ability to use sulphate as the sole source of sulphur to an absolute requirement for cystine and methionine as well as for one or more of the sulphur containing vitamins". Volkonsky (1938) has classified the organisms into two categories : (*i*) "Euthiotrophe", which can obtain sulphur from "$SO_4^{=}$" ions and (*ii*) "Parathiotrophe", which cannot utilize "$SO_4^{=}$" ions.

The present paper deals with the effect of temperature and different carbon and sulphur sources on the growth and sporulation of some members of Sphaeropsidales.

MATERIALS AND METHODS

Four species of Sphaeropsidales, *viz.*, *Botryodiplodia* sp., *Botryodiplodia theobromae* Pat., *Diplodia cajani* Raychaudhuri and *Macrophomina phaseoli* (Maubl.) Ashby were taken up for the present study. The cultures of *Botryodiplodia theobromae* Pat. and *Macrophomina phaseoli* (Maubl.) Ashby were provided by the Head of the Mycology Section, I. A. R. I., New Delhi, and the rest were obtained from Dr. J. C. Edward, Agriculture Institute, Naini (Allahabad). The basal medium consisted of glucose 5·0 gms., asparagine 2·0 gms., $MgSO_4$. $7H_2O$ 0·5 gm., K_2HPO_4 0·5 gm. and distilled water 1000 c.c. To study the effect of temperature on the growth of the fungi under investigation the basal medium was solidified by adding 20 gms. agar. It was then autoclaved at 15 lbs. pressure for 15 minutes. Sterilized Petri dishes of equal size (10 cm. in diameter) were taken and equal quantity of the medium (10 c.c.) was poured in each Petri dish. After incubating them at room temperature (mean 25°C) for three days the contaminated ones were rejected. The remaining dishes were then kept at a temperature, at which the growth was to be observed, at least for three hours before inoculation to remove the lag effect. The various temperatures tried were 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, and 55°C. The dishes were inoculated with the fungi under experiment, keeping the size of the inoculum equal (4 mm. in diameter) in all cases.

All the experiments were run in triplicate. The measurements for radial growth were taken at the end of 24 hours and 48 hours. Observations were made along two lines A and B cutting at right angles to each other, the seat of inoculum being the centre. The mean of the two diametric readings was calculated. The thermal death point of the mycelium was determined by keeping some of the inoculated dishes for 12 to 36 hours, at temperatures on which it did not grow. The dishes were then transferred to room temperature. The failure of the fungus to grow indicated that death has taken place and that the temperature to which, the fungus was exposed, was its thermal death point.

To study the effect of different carbon sources on the growth of the above fungi, glucose of the basal medium was replaced by different carbohydrates sugar alcohols and organic acids, singly, in quantities so as to furnish 2000 mgs. of carbon per litre. While studying the effect of different inorganic and organic sulphur sources on the growth of these fungi $MgSO_4$ of the basal medium was replaced by various sulphur compounds so as to furnish 135 mg. of sulphur per litre. The rest of the procedure including the statistical analysis of the data, was the same as outlined in an earlier paper from this laboratory (Saksena and Kumar, 1961).

Chemicals of high grade purity (Analar of B. D. H. and E. Merck) were used. The cultures of these fungi were maintained on a medium containing peptone 1 gm., dextrose 2·5 gms., distilled water 1000 c.c. and agar 20 gms. at 25°C.

EXPERIMENTAL

(*a*) *Effect of Temperature* :

The results obtained on the effect of temperature on the growth of *Botryodiplodia* sp., *Botryodiplodia theobromae*, *Diplodia cajani* and *Macrophomina phaseoli* are summarized in Table 1.

TABLE I

Showing the diametric spread (in cms.) of the fungal colonies and thermal death point of the four fungi under study

Temp.	Time in hrs.	Diametric spread (in cms.)			
		Botryodiplodia sp.	*B. theobromae*	*Diplodia cajani*	*Macrophomina phaseoli*
10°C	1–24	No growth	No growth	No growth	No growth
	24–48	No growth	No growth	No growth	No growth
15°C	1–24	Negligible	0·2 cm	0·2 cm	Negligible
	24–48	0·5 cm	0·8 cm	0·9 cm	0·6 cm
20°C	1–24	1·8 cm	0·8 cm	1·5 cm	Negligible
	24–48	1·7 cm	1·0 cm	1·4 cm	0·8 cm
25°C	1–24	2·1 cm	1·5 cm	1·9 cm	1·0 cm
	24–48	2·2 cm	2·3 cm	2·0 cm	1·8 cm
30°C	1–24	5·3 cm	3·6 cm	4·0 cm	4·5 cm
	24–48	3·5 cm	5·2 cm	3·4 cm	4·4 cm
35°C	1–24	3·4 cm	2·4 cm	2·2 cm	2·0 cm
	24–48	3·6 cm	4·6 cm	2·8 cm	5·9 cm
40°C	1–24	Negligble	Negligible	0·3 cm	2·0 cm
	24–48	0·3 cm	0·3 cm	0·3 cm	0·2 cm.
45°C	1–24	No growth	No growth	Negligible	No growth
	24–48.	No growth	No growth	0·3 cm	No growth
50°C	1–24	No growth	No growth	Negligible	No growth
	24–48	No growth	No growth	Negligible	No growth
55°C	1–24	No growth	No growth	No growth	No growth
	24–48	No growth	No growth	No growth	No growth
Thermal Death Point		Between 12 and 18 hrs. at 53°C	Between 12 and 18 hrs. at 50°C	Between 18 and 24 hrs. at 55°C	Between 6 and 12 hrs. at 52°C

It is evident from the table that the minimum temperature for the growth of these fungi lies between 10°C and 15°C. Growth of the present organisms increased with the increase in temperature upto 30°C. A fall in the rate of growth of these organisms with the further increase in temperature indicates that 30°C is the optimum temperature for the growth of the fungi under investigation (Figs. 1, 2, 3 and 4). Except for *Macrophomina phaseoli*, which grew well at 40°C, there was very little growth in the case of rest of the fungi. At 45°C and 50°C only *Diplodia cajani* showed some growth while the rest failed to grow. At 55°C even this fungus did not grow.

None of the above fungi showed a constant rate of growth at one temperature. *Botryodiplodia* sp., grew faster during the first 24 hours while *Botryodiplodia theobromae* did so in the second 24 hours. *Diplodia cajani* and *Macrophomina phaseoli* showed a general tendency of growing faster in the second 24 hours, though at 20°C and 30°C growth of *Diplodia cajani* was faster during first 24 hours.

Thermal death point was found to be different for the four fungi investigated. *Botryodiplodia* sp. tolerated 53°C upto 12 hours but died after being kept for

18 hours at this temperature. *Botryodiplodia theobromae* failed to grow at room temperature when it was removed from the incubator at 50°C after 18 hours. Death of *Diplodia cajani* occured at 55°C between 18 and 24 hours while *Macrophomina phaseoli* died at 52°C between 6 and 12 hours.

(*b*) *Carbon requirements* :

The results are tabulated in table 2 for *Botryodiplodia* sp., *Botryodiplodia theobromae*, *Diplodia cajani* and *Macrophomina phaseoli*.

TABLE 2

Showing the average dry weight in mgs. of four fungi on different carbon sources

Carbon sources	*Botryodiplodia* sp.	*B. theobromae*	*Diplodia cajani*	*Macrophomina phaseoli*
1. L-Arabinose	43	12	36	10
2. D-Xylose	4	4	9	5
3. Glucose	19	13	13	25
4 Mannose	24	6	10	35
5. Sucrose	31	23	13	25
6. Maltose	30	25	11	36
7. Starch	16	11	13	25
8. Dextrin	35	18	17	33
9. Mannitol	11	4	12	6
10. Sorbitol	33	18	16	16
11. Glycerol	12	–	5	24
12. Malic acid	6	–	3	10
13. Tartaric acid	6	3	20	3
14. Control	–	–	–	4
Average	19·309	9·71	12·71	18·35

For statistical calculations the results of an individual organism on different sources of carbon were compared.

Summary of dry weight results and conclusions at 1% level of P for *Botryodiplodia* sp.

Treatments	Highly significant
Replicates	Non-significant
S. E.	0·532
C. D. at 1% level	±2·087

Dry weight results :

Nos.	1	8	10	5	6	4	3	7	11	9
Dry weight in mgs.	43	35	33	31	30	24	19	16	12	11

12	13	2	14
6	6	4	0

Summary of dry weight results and conclusions at 1% level of P for *Botryodiplodia theobromae.*

Treatments	Highly significant
Replicates	Non-significant
S. E.	0·51
C. D. at 1% level	±1·993

Dry weight results :

Nos.	6	5	10	8	3	1	7	4	9	2	12	11	12	14
Dry weight in mgs.	25	23	18	18	13	12	11	6	4	4	3	0	0	0

Summary of dry weight results and conclusions at 1% level of P for *Diplodia cajani.*

Treatments	Highly significant
Replicates	Non-significant
S. E.	0·339
C. D. at 1% level	± 1·563

Dry weight results :

Nos.	1	13	8	10	7	3	5	9	6	4	2	11	12	14
Dry weight in mgs.	36	20	17	16	13	13	13	12	11	10	9	5	3	0

Summary of dry weight results and conclusions at 1% level of P for *Macrohomina phaseoli.*

Treatments	Highly significant
Replicates	Non-significant
S. E.	0·408
C. D. at 1% level	± 1·598

Dry weight results :

Nos.	6	4	8	5	3	7	11	10	1	12	9	2	14	13
Dry weight in mgs.	36	35	33	25	25	25	24	16	10	10	6	5	4	3

A careful review of the table 2 shows that except for *Botryodiplodia theobromae* which could not utilize glycerol and malic acid for its growth, all other fungi could grow on all the sources used in the present investigation. None of these fungi showed any growth on carbon free media excepting *Macrophomina phaseoli* which showed negligible growth on it.

Amongst the pentoses, arabinose proved to be a good source for *Botryodiplodia* sp., *Botryodiplodia theobromae* and *Diplodia cajani*, while for *Macrophomina phaseoli* it was a poor source. Xylose, on the other hand, was a poor source for the growth of all the organisms studied. Out of the two hexoses, dextrose supported good growth of *Botryodiplodia theobromae* and *Macrophomina phaseoli*. For the rest of the fungi it was only a mediocre source. Mannose proved to be good for the growth of *Botryodiplodia* sp. and *Macrophomina phaseoli* and for the rest of the fungi it was a poor source.

The disaccharides, *viz.*, sucrose and maltose supported good growth of *Botryodiplodia* sp., *Botryodiplodia theobromae* and *Macrophomina phaseoli* while for *Diplodia cajani* they were mediocre sources.

Soluble starch was a good source for the growth of *Macrophomina phaseoli*, mediocre for *Botryodiplodia theobromae* and *Diplodia cajani* and poor for *Botryodiplodia* sp. Dextrin, on the other hand, supported good growth of all the four fungi studied.

Of the three sugar alcohols tried, glycerol proved to be a good source for the growth of *Macrophomina phaseoli* but was a poor source for the rest. Sorbitol was a good source for the growth of all the organisms except *Macrophomina phaseoli* which utilized it poorly. Mannitol was a poor source for all the fungi studied except *Diplodia cajani* for which it was a mediocre source.

All the organic acids tried in the present investigation *viz.*, malic acid and, tartaric acid, supported poor growth of these organisms except *Diplodia cajani* where tartaric acid proved to be a good source.

(*c*) *Sulphur requirements* :

The dry weight results of *Botryodiplodia* sp., *Botryodiplodia theobromae*, *Diplodia cajani* and *Macrophomina phaseoli*, obtained on different sulphur sources, are presented in table 3.

TABLE 3

Sulphur compounds	Dry weight in mgs.			
	Botryodiplodia sp.	*B. theobromae*	*Diplodia cajani*	*Macrophomina phaseoli*
1. Potassium sulphate	35	30	39	40
2. Sodium sulphite	—	—	41	—
3. Sodium hyposulphite	—	34	35	40
4. Sodium thiosulphate	41	33	32	29
5. Sodium bisulphite	—	9	38	42
6. Cystine	—	—	—	—
7. Thio-urea	33	27	24	39
8. Methionine	40	36	35	41
9. Control (No sulphur)	—	15	18	23
Average	37·25	20·46	29·11	28·22

For statistical analysis the results of an individual organism on different sources of sulphur were compared.

Summary of the dry weight results and conclusions at 1% level of P for *Botryodiplodia* sp.

Treatments	Highly significant
Replicates	Non-significant
S. E.	0·251
C. D. at 1% level	± 1·033

Dry weight results :

Nos.	4	8	1	7	2	3	5	6	9
Dry weight in mgs.	41	40	35	33	0	0	0	0	0

Summary of the dry weight results and conclusions at 1% level of P for *Botryodiplodia theobromae.*

Treatments	Highly significant
Replicates	Non-significant
S. E.	0·482
C. D. at 1% level	± 1·985

Dry. weight results :

Nos.	8	3	4	1	7	9	5	2	6
Dry wt. in mgs.	36	34	33	30	27	15	9	0	0

Summary of the dry weight results and conclusions at 1% level of P for *Deplodia cajani.*

Treatments	Highly significant
Replicates	Non-significant
S. E.	0·618
C. D. at 1% level	± 2·516

Dry weight results :

Nos.	2	1	5	3	8	4	7	9	6
Dry weight in mgs.	41	39	38	35	35	32	24	18	0

Summary of the dry weight results and conclusions at 1% level of P for *Macrophomina phaseoli.*

Treatments	Highly significant
Replicates	Non-significant
S. E.	0·47
C. D. at 1% level	± 1·935

Dry weight results :

Nos.	5	8	1	3	7	4	9	2	6
Dry weight in mgs.	42	41	40	40	39	29	23	0	0

A critical examination of the résumé presented in table 3 reveals that potassium sulphate was a good source for all the fungi except *Botryodiplodia* sp., for which it was a poor source. Sodium hyposulphite did not support any growth of *Botryodiplodia* sp., but for the rest it was a good source. Sodium sulphite, which was a good source for the growth of *Diplodia cajani*, did not support any growth for the rest of the organisms in the present study. Sodium bisulphite proved to be a good source for the growth of *Diplodia cajani* and *Macrophomina phaseoli*, poor for *Botryodiplodia theobromae* but did not support the growth of *Botryodiplodia* sp. Sodium thiosulphate was a good source for all the fungi tested. For *Macrophomina phaseoli* it was more than average.

Of the three organic sources tried cystine did not support any growth of any of the fungi under investigation. On the other hand methionine proved to be a good source for all of these fungi. Thio-urea supported good growth of *Bortyodiplodia theobromae* and *Macrophomina phaseoli* but for *Botryodiplodia* sp., and *Diplodia cajani* it was a poor source.

Except for *Botryodiplodia* sp. all other fungi grew in control, although poorly.

DISCUSSION

Growth of a fungus is possible only within a certain range of temperature which differs with different fungi. The minimum temperature for the growth of most of the fungi lies between 0°C to 5°C, though there are several fungi whose minimum temperature is much higher. Ames (1915) working with *Cephalothecium roseum* and Tisdale (1917) with *Fusarium lini* reported that the minimum temperature for the growth of their organisms was 9°C and 10°C respectively. All the fungi used in the present study have a minimum temperature between 10°C and 15°C. Raychaudhuri (1942) found that *Diplodia cajani* could not grow at 15°C or below it.

The optimum temperature (30°C) of present organisms is in accord with that of *Diplodia natalensis* (Fawcett, 1921) *Piricularia* sp. (Ramakrishnan, 1940) and *Fusarium oxysporum* and *F. radicicola* (Edson and Shapolov, 1920).

The maximum temperature for *Diplodia cajani* in the present study is different from that reported by Raychaudhuri (1942) for the same species. This may be due to difference in strains employed in the two studies. According to Lilly and Barnett (1951, p. 33), both the temperature and the medium on which the organisms are grown prove to be of great importance in the determination of growth.

Lilly and Barnett (1951, p. 28) found that the growth of *Aspergillus rugulosus* was not constant when it was grown under constant conditions. These authors believed that 2 factors were mainly responsible for the non-uniform growth of many organisms ; (1) the change in concentrations of nutrients due to diffusion and utilization and (2) the excretion of inhibitory matabolic products into the medium. Moreover, according to these authors, the same fungus may have a constant rate of growth at one temperature and not at other. The above statements were confirmed by the results obtained in the present studies because none of the four fungi grew constantly under constant conditions.

The results of the experiments enumerated in table 2 prove that all the fungi tested are not able to utilize same sugars. In the present studies whereas arabinose supported good growth of majority of fungi, xylose was a poor source for all of them. Although many workers like Farries and Bell (1930), Saksena (1940) and Bhargava (1945) found pentoses to be of little value for the growth of fungi used by them, the results of present investigation lead to the conclusion that all pentoses are not valueless as regards their utilization by fungi. The preferential utilization of arabinose over xylose, in the present studies, confirms the observations made by Tamiya (1932) on *Aspergillus oryzae*, Lilly and Barnett (1953) on *Endoconidiophora adiposa*, *E. virescens*, *Monilia fructicula* and *Sphaeropsis malorum*, Bilgrami (1956) on *Phyllosticta cycadina P. artocarpina* and *Pestalotia mangiferae* and Kumar (1962) on some species of *Penicillium*.

Dextrose and mannose, the two hexoses, did not prove to be of the same value for the growth of all the fungi under investigation, although these compounds have the same configuration. Lilly and Barnett (1951, p. 120) write that glucose is utilized by more fungi than any other sugar and is nearly a universal carbon source. The behaviour of *Botryodiplodia* sp. and *Macrophomina phaseoli*, in the present studies, is similar to that of *Penicillium digitatum* (Fergus, 1952), *Ustilago zeae* (Wolf, 1953), *Curvularia penniseti* (Agarwal, 1958), and *Phyllosticta cycadina*, *P. artocarpina* and *Pestalotia mangiferae* (Bilgrami, 1956) for the favourable utilization of mannose. However, *Botryodiplodia theobromae* and *Diplodia cajani* resembled other organisms studied by Horr (1936), Bhargava (1945) and Mehrotra (1951) in showing poor response towards this compound.

The results regarding the favourable utilization of sucrose and maltose by *Botryodiplodia* sp., *B. theobromae* and *Macrophomina phaseoli* are in accord with those obtained by Herrick (1940), Blank and Talley (1941), Cantino (1949), Wolf (1953), Bilgrami (1956) and Raizada (1957) for different fungi studied by these authors.

According to Lilly and Barnett (1951, p. 133 and 135) polysaccharides, in general, are insoluble or only colloidally soluble. The utilization of these substances by fungi depend upon the secretion of the necessary hydrolytic enzymes. Thus only those fungi which produce amylase are able to utilize starch. The enzymatic hydrolysis of starch may be represented schematically as follows: Starch → dextrin → maltose → D-glucose. In the present studies starch supported good growth of *Macrophomina phaseoli*, moderate of *Botryodiplodia theobromae* and poor of *Botryodiplodia* sp. On dexrtin, however, all the organisms of present study showed good growth. This behaviour of different fungi, towards the utilization of starch, is probably due to relative effectiveness of amylase for hydrolysing starch to its next hydrolytic product, dextrin.

Reduction of the sugars yields sugar alcohols, *e.g.*, D-sorbitol arises by the reduction of D-glucose and D-mannitol by the reduction of D-fructose or D-mannose. It has been observed (Perlman, 1950 ; Mehrotra, 1951 and Fergus, 1952) that many fungi, with generally wide substrate ranges, grow only poorly or not at all with mannitol as sole source of carbon. The poor utilization of mannitol by the majority of fungi of present investigation confirm the above statement. Sorbitol, a good source for the growth of most of the organisms under investigation, is a redution product of glucose and hence well utilized. It has been stated by Cochrane (1958) that the ability to utilize glycerol appears to be specific in the fungi. Thus the poor utilization of this compound by *Botryodiplodia* sp., *B. theobromae* and *Diplodia cajani* can be compared with the organisms studied by Tochinai (1926) Tamiya (1932), Steinberg (1942) and Agarwala (1955). *Macrophomina phaseoli* however, resembled, in its behaviour of favourable utilization of this compound

with *Colletotrichum phomoides* (Kendrick and Walker, 1948) *Memnoniella echinulata* (Perlman, 1948) and *Glaeosporium musarum* (Grewal, 1954).

The statement of Leben and Kiett (1948) that organic acids are poor sources of carbon for the growth of *Venturia inequalis* finds further support from the present study where the organic acids are generally poor sources. The results regarding the favourable utilization of tartaric acid by *Diplodia cajani* are in confirmity with those obtained by Beckman *et al.* (1953) and Agarwal (1955).

It has been shown by Steinberg (1936), Barner and Cantino (1952) and Hungate and Mannell (1952) that at moderate carbohydrate concentrations the sulphur requirements of most of fungi is about 0·0001 – 0·0005 M. As a large part of this much amount of sulphur is generally present as impurities even in pure chemicals growth of the fungi is generally observed in sulphur free media, as is the case in the present study.

According to the Lilly and Barnett (1951, p. 92) sulphate sulphur is most common source of sulphur used in the media. This source was favourably uitlized by most of the present fungi and thus they lend further support to the results obtained by Armstrong (1921), Mosher *et al.* (1936), Tandon (1950) and Agarwala (1955). The results of good growth of the present organisms, except *Botryodiplodia theobromae* on bisulphite sulphur (given in the form of sodium bisulphite), are in accord with those of Agarwala (1955) and similarly the results of present fungi except *Diplodia cajani* in the poor utilization of sodium sulphite agree with those obtained by Bhargava (1945) for fungi studied by him. The behaviour of the two exceptions, cited above, can be compared with those studied by Tandon (1950) and Agarwala (1955) respectively. Further, both sulphite and bisulphite were found to be toxic for *Botryodiplodia theobromae* ; while the former did not support any growth of this organism, the latter, acting as a toxin, gave growth even less than that obtained on the control. These results agree with those obtained by Steinberg (1941) for *Aspergillus niger.*

Majority of the fungi in the present investigations could be classed with *Brevilegina gracilis* (Bhargava, 1945) for favourable utilization of sodium hyposulphite. In the utilization of sodium thiosulphate all the species except *Macrophomina phaseoli* resembled with the fungi investigated by Steinberg (1941), Bhargava (1945) Mehrotra (1949), Saksena *et al.* (1952) and Agarwala (1955), who reported good growth of their respective fungi on this source.

Lilly and Barnett (1951, p. 92) state that amongst the organic compounds structure is enormously important and the specific structure of organic sulphur compounds effects utilization. Of the three organic compounds used in the present study, cystine did not support any growth of any of the fungi tested. On the other hand, methionine was a good source for all of them. According to Fruton and Simmonds (1958) methionine is an indispensable amino acid for all animals that have been investigated, while cystine and cysteine are dispensable, as the sulphur of methionine can be used in the biosynthesis of cystine. Similarly Fling and Horowitz (1951) found that *Neurospora* can convert methionine to cystine. It is presumed here that cystine is dispensable for the growth of present organisms.

Good growth shown by *Botryodiplodia theobromae* and *Macrophomina phaseoli* on medium containing thio-urea indicated that these fungi could make use of the sulphur attached to the carbon and as such these fungi resembled with *Penicillium notatum* (Challenger and Liu, 1950) and *Phytophtora cactorum* (Mehrotra, 1949). However, in the poor utilization of this compound, *Botryodiplodia* sp. and *Diplodia cajani* resembled with fungi studied by Steinberg (1941), Agarwala (1955) and Bilgrami (1956).

SUMMARY

The effect of temperature and different carbon and sulphur sources on the growth of four members of Sphaeropsidales, *viz.*, *Botryodiplodia* sp., *B. theobromae*, *D. cajani* and *M. phaseoli* was noted. The minimum temperature was between 10°C and 15°C while the optimum temperature was 30°C. The upper limit of temperature range was 40°C for *Botryodipladia* sp. and *B. theobromae* and 45°C and 50°C for *Macrophomina phaseoli* and *Diplodia cajani* respectively. The rate of growth of these organisms increased with the rise of temperature till 30°C. None of the present fungi showed a constant rate of growth under constant conditions.

Amongst the pentoses (carbon sources) arabinose was preferentially utilized over xylose. Of the hexoses dextrose was good source for *Botryodiplodia theobromae* and *Macrophomina phaseoli* while mannose was good for *Botryodiplodia* sp. and *Macrophomina phaseoli*. For the rest of the organisms dextrose was moderate and mannose was a poor source of carbon. The disaccharides—sucrose and maltose—were good sources for most of the organisms studied. Of the two polysaccharides dextrin was good for all the present fungi while starch varied from good to poor for different fungi investigated. Sorbitol was good for the growth of majority of the fungi but mannitol and glycerol were poor sources for most of them. Except for *Diplodia cajani* which grew well on tartaric acid, none of the present fungi showed any liking either for malic or tartaric acid.

Out of the inorganic sulphur sources tried potassium sulphate and sodium hyposulphite were good sources for all the fungi except *Botryodiplodia* sp., but sodium sulphite was good only for *Diplodia cajani*. Sodium thio-sulphate supported good growth of majority of present organisms. However, sodium bisulphite proved to be good for *Diplodia cajani* and *Macrophomina phaseoli* only. Amongst the organic sulphur compounds cystine did not support any growth of these fungi, thio-urea was a poor source for *Botryodiplodia* sp. and *Diplodia cajani* and methionine was good for all of them.

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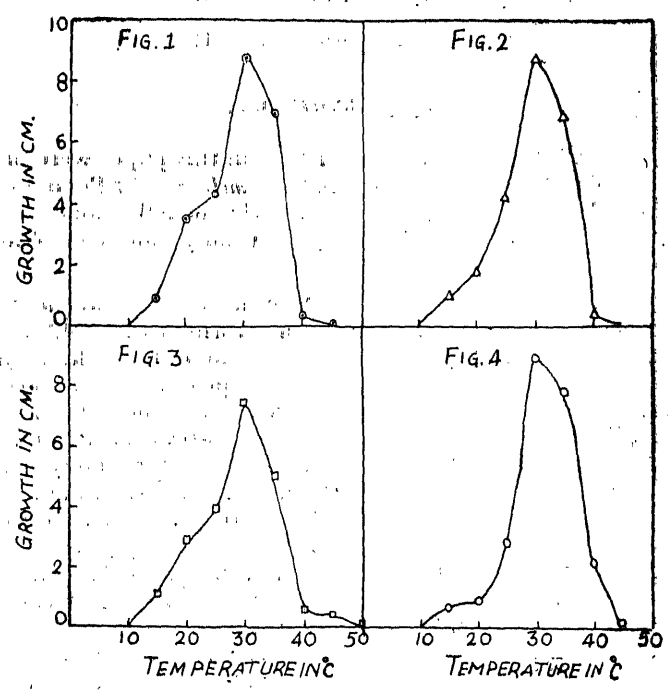

Figs. 1, 2, 3, 4 : Showing the growth curves at different temperatures of *Botryodiplodia* sp., *Botryodiplodia theobromae* Pat., *Diplodia cajani* Raychoudhuri and *Macrophomina phaseoli* (Maubl.) Ashby respectively.

TWO NEW SPECIES OF THE GENUS *TYLODELPHYS* DIESING, 1850 (TREMATODA : DIPLOSTOMIDAE POIRIER, 1886) FROM THE INDIAN SNAKE BIRD *ANHINGA MELANOGASTER* PENNANT.

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Tylodelphys darteri, n. sp.

The two species of trematodes described in this paper were obtained from the small intestine of common snake-bird *Anhinga melanogaster* Pennant, 1769. The birds were either shot or caught in the subarbs of Allahabad. A number of specimens of *T. darteri*, n. sp., were obtained from only one out of about twenty birds examined.

The living distome in salt solution is white in colour and shows slow movements of contraction and expansion. The mature worm is 1·82–2·42 m.m. in length and 0·71–0·99 m.m. in maximum breadth in the region of the holdfast organ. It is indistinctly divided into flattened, more on less pear shaped anterior body and conical posterior body. The anterior body with a concavity on the ventral surface contains all the adhesive organs and measures 0·99–1·25 m.m. in length and 0·735–0·945 m.m. in maximum breadth. The conical hind body is smaller than the forebody measuring 0·87–1·13 m.m. in length and 0·615–0·72 m.m. in maximum breadth which occurs in the region of the anterior testis. The anterior end of the fore-body is slightly trilobed, the median lobe being slightly protruded and bears the oval oral sucker measuring 0·066–0·082 m.m. in longitudinal and 0·089–0·099 m.m. in transverse axis. The side lobes form the two flanks, each having a pseudo-sucker which extends a little in front of the middle of oral sucker, anteriorly and a little behind the long axis of pharynx posteriorly. Pseudosuckers measure 0·125–0·175 m.m. in length and 0·05–0·1 m.m. in maximum breadth.

A prepharynx is absent. The pharynx, which directly follows the oral sucker, is not very muscular and measures 0·079–0·082 m.m. in length and 0·056–0·066 m.m. in breadth. The Oesophagus is very small, 0·083 m.m. in maximum length and 0·026 m.m. in maximum breadth, and it bifurcates into the intestinal caeca at 0·228 m.m. behind the anterior extremity of the body. The narrow intestinal caeca, 10–19 μ in width, run laterally 0·0363–0·0693 m.m. away from the ventral sucker, pass very close to the holdfast organ and are covered dorsally by the vitelline follicles. Due to the crowding of the genitalia it is rather difficult to pursue the course of the caeca posteriorly beyond the holdfast organ. The ventral sucker, as in all the other known species of the genus, is smaller than the oral sucker, transversely elongated, measuring 0·066–0·073 m.m. in length and 0·089–0·109 m.m. in breadth and lying 0·54–0·6 m m. behind the anterior end of the body. The almond shaped holdfast organ with a narrow longitudinal slit and situated 0·066–0·075 m.m. behind the hinder margin of the ventral sucker is always elongated. It measures 0·24–0·33 m.m. in length and 0·199–0·294 m.m. in breadth.

The testis as usual are post ovarian, tandem, lie very close to each other, filling almost the entire width of the hind body. They are separated from each other in the middle by a maximum distance of 0·66 m.m. They are almost symmetrical in shape and are broader than long. The anterior testis, with a ventral, concavity, measures 0·195–0·24 m.m. longitudinally and 0·555–0·645 m.m. transversally. The posterior testis almost of similar shape as the anterior, measures 0·345–0·48 m.m. in length and 0·465–0·525 m.m. in width and it lies 0·248–0·33 m.m. distance in front of the posterior extremity of the body. The large vesicula seminalis situated very near the posterior testis is coiled and passes terminally into the narrow ductus ejaculatorious. The latter unites with the terminal part of the uterus to form the hermaphroditic canal which runs through the well develeped muscular genital cone inside the bursa-copulatrix.

The ovary is situated on the left side dorsally at the junction of the anterior and posterior body with its major portion in the former body part. It is pear shaped, placed obliquely, with its rounded posterior end very near the anterior testis and the pointed anterior end reaching the middle of the body, close behind the holdfast organ. The ovary measures 0 125–0·165 m.m. in length and 0·182–0·2 m.m. in width. The oviduct arises from the antero-ventral side of the ovary, proceeds backwards and in between the two testes in the median region forms the ootype where it is surrounded by the shell gland mass ventrally and covered by yolk reservoir dorsally. The uterus at first runs forward to the left side upto the holdfast organ, *i.e.*, for some distance even in the fore-body, finally after making a loop it turns back as a straight median tube to open into the genital cone. The genital cone is quite large and it almost fills the genital atrium or bursa-copulatrix. There is no body constriction separating the genital atrium. The genital opening is dorsally situated, 0·033–0·066 m.m. in front of the posterior extremity.

The vitellaria consisting of numerous pear-shaped follicles extend from a little in front of the ventral sucker forming four distinct bands two on each side of the body. The vitelline bands unite in the hind body just before the anterior testis and form a median ribbon in the testicular region which again divides behind the posterior testis and the two vitelline bands run laterally to surround the vesicula seminalis but leave the bursa copulatrix uncovered. The vitelline follicles are more numerous in the anterior body, surround the holdfast organ partly overlapping it and get mixed up with the adhesive gland which is situated within the latter organ marginally. The yolk reservoir lies median in between the two testes. The number of ova in the uterus does not exceed five and each ovum measures 94×56 μ in size.

DISCUSSION

Tylodelphys darteri, n. sp. resembles the other species of the genus in having the body indistinctly divided in two parts, trilobed anterior body end having oral sucker in the central lobe and pseudosuckers on the side lobes, hold-fast organ of ovoid shape, topography of the genital organs, presence of bursa copulatrix and in the position of the genital opening. It differs from all of them in the general shape of the body and the size of the testes which are larger than those in the known species. In general shape of the body the new species comes very close to *Tylodelphys americana* Dubois, 1937 but it differs from it in the absence of prepharynx, size and shape of the holdfast organ, extent and distribution of vitellaria and absence of body constriction limiting the extension of the bursa copulatrix. The new species also differs in the measurements of its various organs from *americana*.

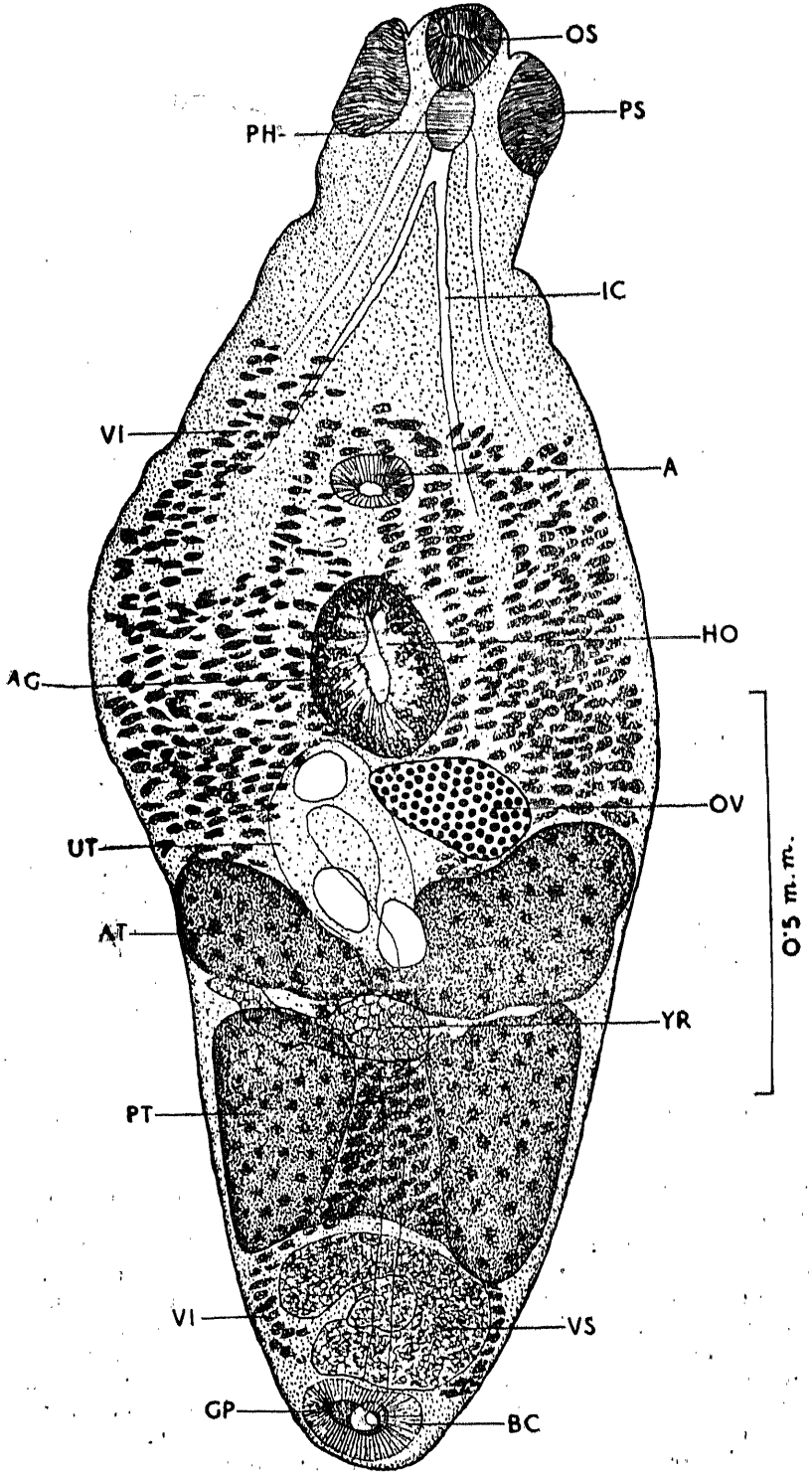

Fig. 1. Ventral view of *Tylodelphys darteri* n. sp.

KEY TO LETTERING USED IN FIGURES

A, acetabulum ; AG, adhesive gland ; AT, anterior testis ; BC, bursa copulatrix ; GP, genital pore ; HO, holdfast organ ; IC, intestinal caecum ; OS, oral sucker ; OV, ovary ; PH, pharynx ; PT, posterior testis; PS, pseudo-sucker; UT, uterus; VI, vitellaria; VS, vesicula seminalis ; YR, yolk reservoir

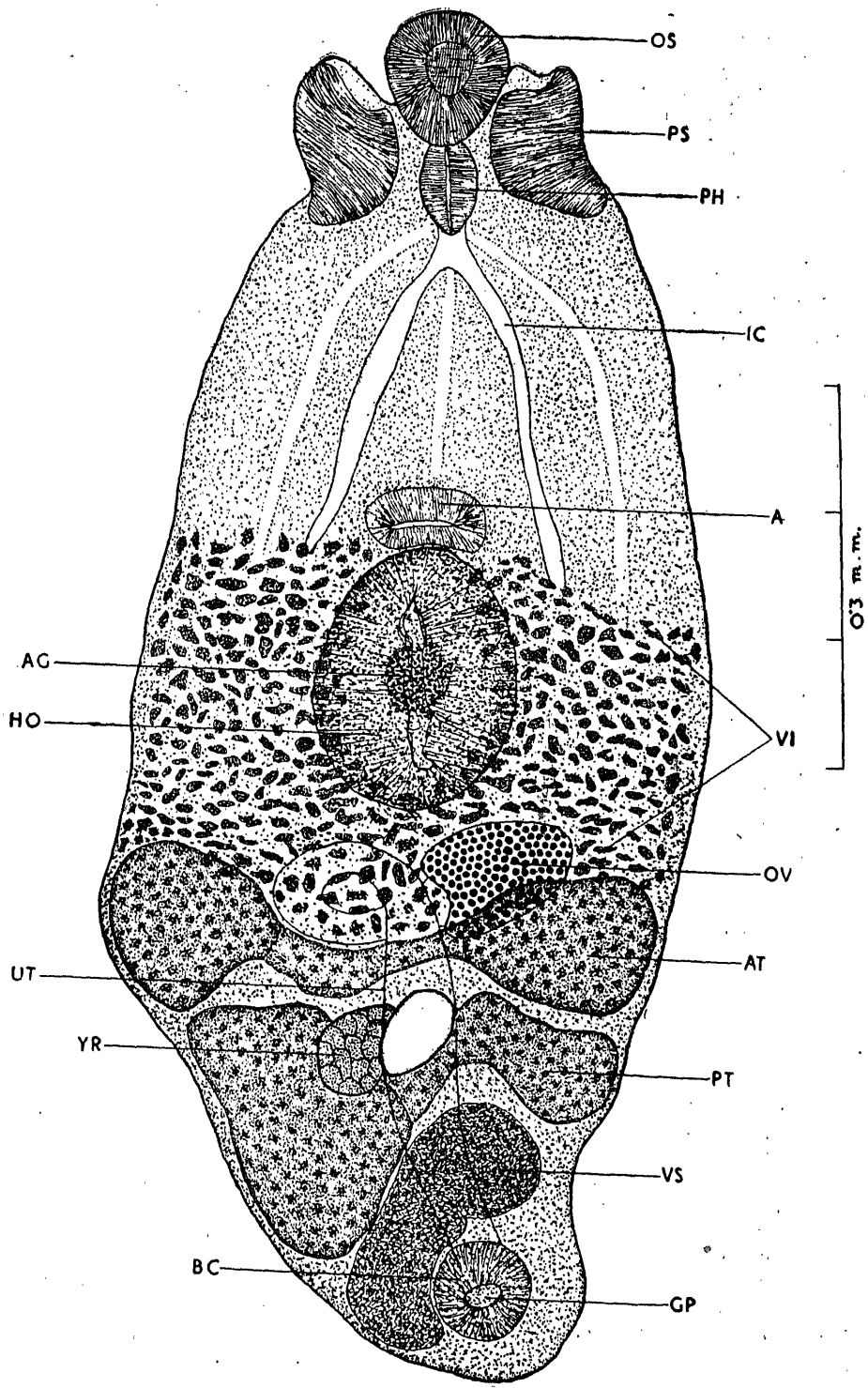

Fig. 2. Ventral view of *Tylodelphys indiana* n. sp.

Lettering as in fig. 1

Tylodephys darteri, n. sp., differs from *T. clavata* Diesing, 1850 in the size and shape of the body and the size of various internal organs, the latter being very small in size. In new species the prepharynx is absent while in *clavata* it is present though small in size. The distribution of vitellaria in the fore-body is quite similar, but in the posterior body behind the testis, the two bands do not surround the vesicula seminalis as in the new species.

The Indian species is altogether different from *T. conifera* Dubois, 1937 being much larger in size, difference in shape of the body and sizes of various internal organs. The shape of the holdfast organ and the extent of vitellaria are so much different that the two species can be immediately separated at a glance. *Tylodelphys darteri*, n. sp. comes much closer to *T. excavata* Szidat, 1935 than any other known species. It resembles *excavata* in size of the body and of the various other internal organs, shape of the holdfast organ, absence of prepharynx and body constriction which separates the bursa copulatrix and limits its extent. But the new species differs from the old one in the shape of the body, extent and distribution of vitellaria in the fore-body and in the size of testes which are larger in *darteri* than in *excavata*.

Host : *Anhinga melanogaster* Pennant, 1769.

Habitat : Small intestine.

Locality : Allahabad, India.

Tylodelphys indiana n. sp.

Several specimens of this species were obtained from the small intestine of an Indian darter *Anhinga melanogaster* caught at Phulpur near Allahabad. In the living condition the distomes are white in colour and show movements similar to the preceding species. As the worms are very small in size and slack in their movements, they were collected after great search.

The sexually mature worms measure 0·95–1·08 m.m. in length and have the body indistinctly divided into two parts. The tongue shaped fore-body, 0·55-0·6 m.m. in length and 0·45 m.m. in maximum breadth in its posterior region, has the cuticle armed with very minute backwardly directed spines. The spines are so small that they give the body surface a serrated appearance under higher magnifications. The conical hind body is shorter both in length and breadth than the fore-body, measures 0·395–0·45 m.m. in length and 0·4–0·45 m.m. in maximum breadth in the region of the anterior testis. The ratio between the length of the fore-body and the hind body is approximately 4 : 3. The anterior end of the body is clearly trilobed, the middle lobe is anterior, somewhat protruded and bears the rounded oral sucker 0·0825–0·1 m.m. in diameter ; the two side lobes bearing the pseudosuckers, one on each side form the two flanks. The well developed muscular pseudosuckers begin behind the anterior one fourth of the oral sucker, extend a little behind the pharynx and measure 0·1–0·109 m.m. in length and 0·076–0·0825 m.m. in breadth. Prepharynx is absent. The oral sucker is immediately followed by the musuclar pharynx which is smaller in size than the oral sucker, oval in shape, measuring 0·07–0·073 m.m. in length and 0·0396–0·042 m.m. in maximum breadth. The Oesophagus is very small 0·0165–0·0231 m.m. in length and it bifurcates into the intestinal caeca which are quite narrow, measuring 0·007–0·0132 m.m. in width. The intestinal caeca are seen only in the fore-body, they run laterally very near outside the ventral sucker 0·0231–0·0363 m.m. away from it and nearly touching the holdfast organ. The transversely elongated oval ventral sucker lies 0·3152–3·36 m.m. behind the anterior end very close to or nearly touching the holdfast organ and measures 0·0462–0·0495 m.m. in length

and 0·1–0·109 m.m. in maximum breadth. The holdfast organ is longitudinally oval with a narrow irregular median slit, situated close behind the ventral sucker and a little in front of the posterior margin of the fore-body, measuring 0·198–0·215 m.m. in length and 0·132–0·148 m.m. in maximum breadth. The adhesive gland forms a dorsal central mass on the holdfast organ.

The testes are tandem, post-ovarian, broader than long, occupying almost the entire width of the hind-body. The horse-shoe shaped anterior testis is always symmetrical with the median concavity in the narrow middle band and measures 0·066–0·129 m.m. in length in the lateral extremities, 0·033–0·04 m.m. in length in the middle narrow portion and 0·345–0·375 m.m. in breadth. The asymmetrical posterior testis situated 0·0495–0·0629 m.m. behind the anterior testis is also curved with a median concavity. Its right arm measuring 0·132–0·215 m.m. is much longer than the left one which measures 0·076–0·0825 m.m. in length and the breadth of the entire posterior testis is 0·315–0·345 m.m.

The ovary is dorsally situated on the left side at the junction of the fore and hind body, a little behind the holdfast organ and slightly overlapping the anterior testis. It is transversely elongated, elliptical or triangular in shape measuring 0·0726–0·08 m.m. in length and 0·115–0·122 m.m. in breadth. The oviduct arises from the inner margin of the anterior side of the ovary, runs posteriorly in the median region in between the two testes to join the shell gland complex. The uterus runs forwards upto the anterior margin of the ovary, and then turns backwards more or less as a straight tube towards the genital atrium. It contains only one or two yellow ova measuring 0·0825 m.m. in length and 0·0528 m.m. in maximum breadth. The large coiled vesicula seminalis occupies most of the space between the posterior testis and the genital atrium. The ductus ejaculatorious being very small is not clearly seen; it meets the uterus which after forming a genital cone opens into the bursa copulatrix The dorsally situated bursa copulatrix is not limited by a body constriction and it opens through the genital opening lying 0·0264 m.m. in front of the hinder end of the body.

Numerous vitelline follicles of irregular shape are strongly developed in the anterior body behind the posterior margin of the ventral sucker surrounding and overlapping the holdfast organ. In the hinder body the vitelline follicles are present only in the anterior region in front of the anterior testis. The yolk reservoir, partly overlapping the posterior testis dorsally, lies slightly to the right side.

DISCUSSION

Tylodelphys indiana, n. sp. resembles the other species of the genus in the indistinct division of the body, trilobed anterior end with oral sucker slightly protruding in the centre, shape of the hind body, shape of the holdfast organ, presence of the bursa copulaurix and position of the genital pore. The new species differs from all of them in the anterior limit of vitellaria which do not lie even in the region of acetabulum and in having an asymmetrical posterior testis.

T. indiana comes closer to *T. clavata* Diesing, 1850 and *T. conifera* Dubois, 1937 in body size, though it is slightly bigger than in the latter species. The new species differs from both of them in the absence of prepharynx, posterior extension of the vitellaria, in size of various internal organs and in having ova of smaller size. The shape of the holdfast organ of *clavata* and *indiana* is similar but the distance between the ventral sucker and the former organ varies (in *indiana* the ventral sucker is nearly in contact with the holdfast organ while in *clavata* it is at some distance). In the distance of holdfast organ from the ventral sucker the new species resembles *conifera* but the shape of the holdfast organ is different in

the two species. Only in the absence of prepharynx *indiana* resembles *T. excavata* Szidat, 1935 otherwise it is completely different from it.

Host : *Anhinga melanogaster* Pennant, 1769.

Habitat : Intestine.

Locality : Phulpur near Allahabad, India.

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ON THE DRY-ROT OF 'BEL' FRUITS, (*AEGLE MARMELOS* CORREA)

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INTRODUCTION

The 'Bel' (*Aegle marmelos* Correa, Fam. Rutaceae) tree occurs chiefly in the Indo-Malayan region. It grows wild in the sub-Himalayan tract, in central and south India and also in Burma, but is often cultivated all over India for its religious and several beneficial medicinal properties, especially as curative for diarrhoea and dysentery. The tree flowers during May to July and the fruits start ripening by December but remain on the tree till as late as the next flowering season.

A hitherto unreported dry-rot disease of the 'Bel' fruits involving the decay of internal portion, was observed by Dr. S. N. Das Gupta in stored fruits. Later a number of fresh 'Bel' fruits of different stages of maturity were collected from the tree, brought to the laboratory, cut open and the tissues examined microscopically. These fruits apparently did not show any disease symptom, although in a number of cases hyphae were seen in a state of dormancy. These fungal hyphae, chiefly present in the epicarp and the outer mesocarp tissues, were subsequently cultured on artificial media.

These fresh fruits, when kept in storage for a few days, and those which were obtained from different places of storage, exhibit the characteristic dry-rot symptoms internally (Plate, Figs. 1-3). The fungi associated with the disease were, therefore, isolated from fruits (*i*) obtained directly from the market and (*ii*) of different sizes (1·5″-4·5″ diameter) plucked from the trees, which were surface sterilised, wrapped in alcohol swabbed packing paper and stored for 4-5 days. The fruits, on cutting, exhibited different stages of decay in the pulp and the affected tissues from the different regions were transferred to sterilised Brown's agar medium. The disease symptoms and the fungi appearing in both the sets of fruits were practically of the same type. A more comprehensive investigation was then undertaken with fruits belonging to the second category and the results of pathogen isolation are summarised in table 1.

TABLE 1

Fungi obtained from incubated 'Bel' fruits of different stages of maturity

Number of fruits incubated	Approximate diameter of fruits (in inches)	Number of fruits showing the 'rot' due to				Number of healthy fruits
		Botrytis cinerea	*Aspergillus nidulans*	*Aspergillus niger*	*A. niger* + *B. cinerea*	
20	1·5-2·5	10	0	0	0	10
20	2·5-3·0	8	1	1	0	10
20	3·0-4·5	1	2	6	2	9
20	,,	5	0	1	12	2
20	,,	6	2	4	0	8

PLATE
(Fig. 1–3)

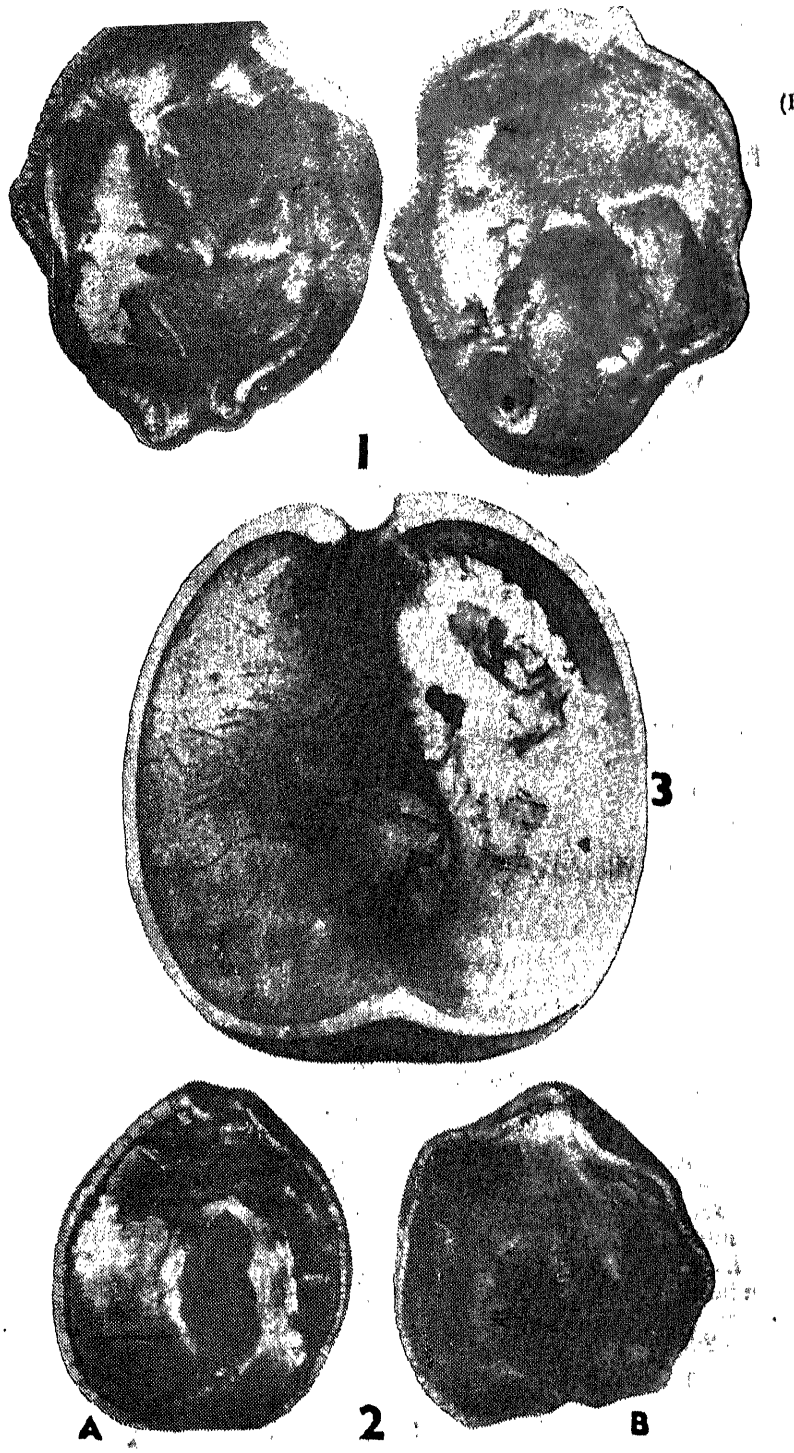


Fig. 1. Young 'Bel' fruits split open to show the advanced stage of dry-rot due to *Botrytis cinerea*. The outer skin is markedly wrinkled while the inside shows the profuse white mycelial growth on the entire pulp.
Fig. 2 Young 'Bel' fruits split open longitudinally to show, (*a*) the spread of the white mycelium of *B. cinerea* all over the pulp involving seed cavities, but the seeds remain unaffected ; (*b*) the developing mycelium of *B. cinerea* from the stalk end region of the 'Bel' fruits in early stages of infection.
Fig. 3. Diagram of the half hard shelled mature 'Bel' fruit showing early stage of the 'black-rot' due to development of *Aspergillus niger* from the stalk end region, gradually spreading over the pulp. At this stage the white mycelium of *B. cinerea* is quite distinct covering nearly half of the tissue towards right.

Three fungi *viz.*, *Botrytis cinerea* Pers. ex Fr., *Aspergillus niger* van Tiegham and *Aspergillus nidulans* (Eidam) Wint. have been obtained in this way. While the aspergilli have been isolated from the older fruits collected during October to May, *B. cinerea* has been found exclusively in young fruits. Of the aspergilli, *A. niger* is more common than *A. nidulans*. These may be present separately or one of them, *A. niger* may be found associated with *B. cinerea* as well. *A. nidulans* and *B. cinerea* have never been observed occurring together.

This, thus, clearly indicates that the disease is a case of latent infection in which the fungal hyphae gain entry while the fruits are borne on the tree and exhibit the disease only in storage.

SYMPTOMATOLOGY

Depending upon the pulp colour developing in the late stages of the dry-rot disease of 'Bel' fruits kept in storage, the symptoms have accordingly been divided into three categories :

(*i*) *Red-rot* :

This disease symptom is found in the fruits of all stages of maturity, but is exclusively present in young soft skinned fruits (1·5"-2·5" diameter) incubated and studied during the period August to October. The healthy pulp at this stage is faint yellow but as the disease advances the entire pulp changes to dark brick red. The pathogen (*Botrytis cinerea*), which has colourless mycelium, develops chiefly from the stalk end region of the fruits and later spreads all over the tissue involving the seed cavities but does not invade the seeds (Plate, Fig. 2). Both the hyphae and spores are hyaline and the characteristic brick red colour, from which the name of the disease is derived, is due to the physiological reaction of the pathogen with the host pulp.

The soft pulp eventually changes into a hard mass containing the unconsumed tissue, hyphal mass and spores. In the final stage the outer rind of the so affected fruits becomes marked by several wrinkles and depressions on the general surface, although it bears the normal healthy green colour even at this stage (Plate, Fig. 1).

(*ii*) *Black-rot* and

(*iii*) *Green-rot* :

These names are also derived from the colour of the diseased pulp at the final stage of the disease. The 'black-rot' is more frequent than the 'green-rot'. Both are found to develop in the hard shelled fruits (3·0"-4·5" diameter) and not in younger stored fruits. The sequence of disease formation in both is the same.

The fungi (*Aspergillus niger* and *Aspergillus nidulans* respectively), associated with these symptoms, also seem to develop from the stalk end region (Plate Fig. 3). These grow comparatively faster and quickly cover the entire golden yellow fleshy pulp. The tissue gradually gets masked by a thick hyphal felt bearing the conidial heads of the infecting fungus. The pulp is subsequently reduced to a shrunken mass, which recedes from the rind while the epicarp shell changes its healthy green colour to dull brown. Finally the pulp is completely consumed and there only remains a hyphal ball bearing the conidial heads of the respective fungal pathogens.

EXPERIMENTAL PRODUCTION OF DISEASE

All the three fungi, *B. cinerea*, *A. niger* and *A. nidulans* isolated from the diseased fruits, were utilised to test their pathogenicity (*a*) on healthy plucked fruits under laboratory conditions and (*b*) on flowers and fruits borne on the tree. The inoculation methods employed were (*i*) placing the inoculum and (*ii*) spraying the spore suspension of the respective fungi on the uninjured and injured surfaces.

(*a*) *Pathogenicity test under Laboratory Conditions* :

A number of fruits, at different stages of maturity were plucked from the trees with their stalks intact. After proper surface sterilisation these were inoculated with the respective pathogen by placing the inoculum on the uninjured and the experimentally injured surfaces, stored in a glass chamber and examined half number from each set after 4 days and the rest after 10 days. Excepting where the infected pulp showed the presence of the inoculated fungus and the invasion of the tissue started from the point of inoculation, other diseased fruits were disregarded. The data are summarised in the table. 2

TABLE 2

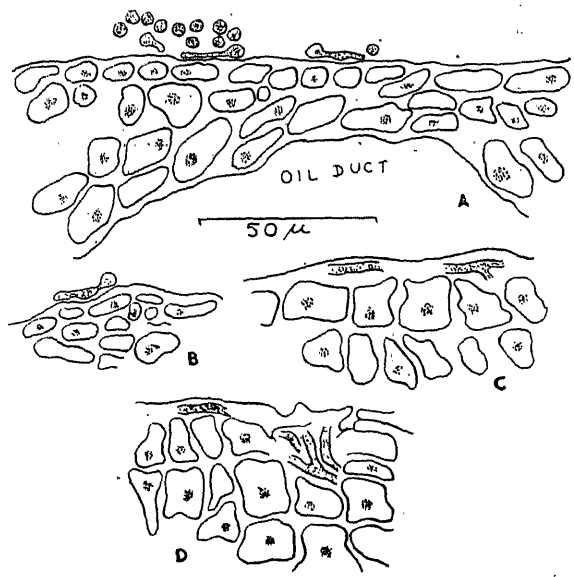
Experimental inoculation of fruits with various fungi

Average diameter of fruits (in inches)	Region of inoculation	Mode of inoculation	Fungus inoculated	Number of fruits inoculated	Number of fruits developing rot	Percentage of fruits developing rot
1·5-2·5 and 3·5-4·5	Stalk-end	Without injury	*B. cinerea*	20	8	40
			A. niger	15	0	0
			A. nidulans	15	0	0
1·5-2·5	,,	With injury	*B. cinerea*	15	12	80
			A. niger	15	6	40
			A. nidulans	15	5	33
3·5-4·5	Stalk-end depression (after removing the stalk)	Without injury	*B. cinerea*	20	0	0
			A. niger	25	13	52
			A. nidulans	25	11	44
,,	,,	With injury	*B. cinerea*	15	10	67
,,	Epicarp	Without injury	*B. cinerea*	15	0	0
			A. niger	15	3	20
			A. nidulans	15	0	0
,,	,,	With injury	*B. cinerea*	20	4	20
			A. niger	20	16	80
			A. nidulnas	20	10	50

All the three fungi proved to be pathogenic. *B. cinerea* secures successful entry through the injured and uninjured stalk end region in fruits of all stages of maturity, but injury favours infection ; whereas the aspergilli can only infect through the wounded stalk end of the young fruits and their usual point of infection being the wounded stalk end depression. The injured epicarp considerably favours the entry of all the pathogens.

(b) Pathogenicity tests on Flowers and Young Fruits 'in Vivo' :

These experiments were commenced when the flowering had just initiated and were continued till the fruits had become 2-3 cm. in average length. Inoculation was done by (*i*) spraying the spores and (*ii*) putting the inoculum. The experimentally infected fruits and flowers were collected from the tree after 10 days and brought to the laboratory. Some of these were examined microscopically but no change was visible internally, while others were utilised to see if (*i*) the disease symptoms developed by keeping them in storage for a week and also (*ii*) if the inoculated pathogen reappeared in 'direct inoculations' and 'moist chamber cultures.' On examination these showed profuse growth of *B. cinerea* and *A. niger* developing below the stalk end of the fruits in which the respective fungi were inoculated round the injured stalk end region. Gradually they seemed to develop over the mesocarp. In all these isolations the frequency of infection due to *B. cinerea* was 70-80%, of *A. niger* 40-60% and of *A. nidulans* 5-8%.



Text Fig. 1. Sections of young 'Bel' fruits experimentally infected with *A. niger*, (*a*) and (*b*) : Thel conidia germinated and as such, attached to the cuticular lining of the fruit. (*c*) : Hypha bits in the subcuticular region. (*d*) Hyphae in the epidermal and subepidermal tissues.

MODE OF INFECTION AND PERENNATION OF THE FUNGUS

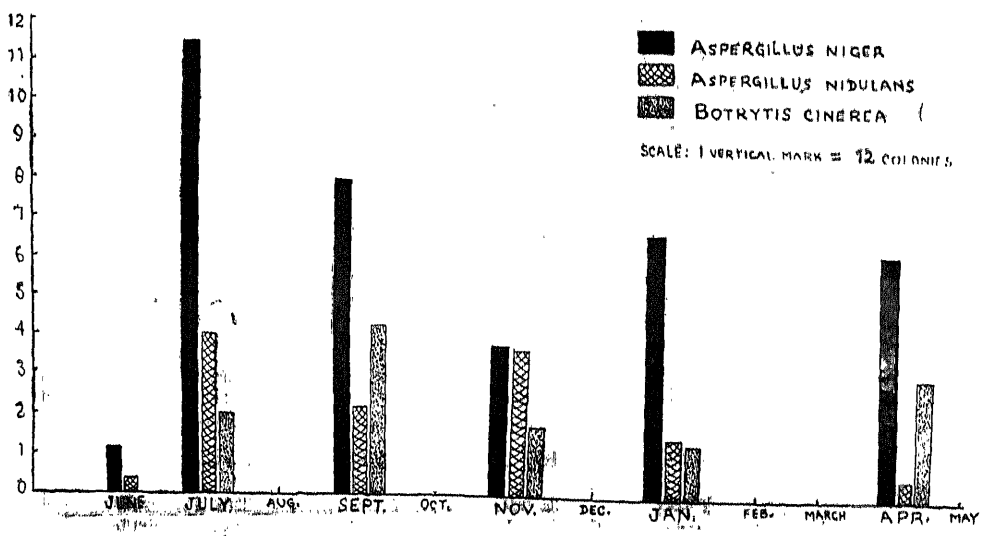
The pathogenicity experiments showed that the pathogens gain entry mainly through the stalk end region of the 'Bel' fruits. *B. cinerea* secures infection through the stalk end, wounded epicarp and the stalk end depression. (Plate Fig. 2). The aspergilli have identical mode of penetration in the fruits through their stalk end depression (Plate, Fig. 3), but can also infect through the epicarp and the stalk end regions if they are injured.

In order to study the precise mode of infection and perennation of the fungi inside the host tissue, microtome sections were studied of the experimentally inoculated flowers (ovary portion) and young fruits collected from the tree after one week of the experimentation. The study showed that a number of conidia of *A. niger* remain firmly attached to the outer ovary wall and the cuticular lining of the

fruits, some of which were in the germinated condition with variable lengths of the germ tube growing along the cuticle (Text Fig. 1, *a* and *b*). Hyphal bits which have been traced in the subcuticular, epidermal and sub-epidermal regions of these experimented fruits (Text Fig. 1, *c* and *d*), indicate that the germ tube gains entry at some place through the cuticle and ramifies in the region lying below it, where they remain dormant as latent infection.

ATMOSPHERIC MYCOFLORA

The atmospheric fungal flora round a tree, during its flowering and fruiting-period, plays an important role in the establishment of the fungi as latent infections, which later on appear as causal pathogens in stored fruits. The study of the atmospheric mycoflora round the 'Bel' tree was made at regular intervals throughout the year, by exposing 16 petri plates containing Brown's agar medium at a height of 10-12 feet for 2 minutes at different corners of the tree. The plates were incubated at 28-30°C, and the colonies appearing after 3-4 days were counted and studied. The observations were made round the year once in two months and the relative frequency of the causal fungi has been represented in text figure 2. These counts indicate that the atmospheric mycoflora consisted of, besides a number of bacteria, seven fungi *viz.*, *Aspergillus niger*, *A. nidulans*, *Botrytis cinerea*, *Fusarium* sp., *Alternaria* spp. *Stemphyllium* sp. and *Penicillium* sp. The occurrence of these fungi in different periods of the year is variable (Text figure 2.)



Text Fig. 2. Showing the relative frequency of *Botrytis cinerea*, *Aspergillus niger* and *A. nidulans* in the atmospheric mycoflora of the 'Bel' tree in different periods of the year.

DISCUSSION

The dry-rot of 'Bel' fruits, essentially a storage disease, reported here for the first time, is caused by three distinct fungi and the characteristic colour of the diseased tissue develops according to the pathogen. The dry-rot has thus been

divided into three types *viz.*, the red-rot caused by *Botrytis cinerea*, black-rot by *Aspergillus niger* and green-rot by *A. nidulans*. These fungi occur as latent infection secured during the developmental period of the fruit on the tree and become active to produce the disease in storage.

A large amount of work has been done on the latent infections in the fruits, both in India as well as abroad. Baker and Wardlaw (1937) demonstrated that, in a number of tropical fruits, fungal spores germinate and establish themselves as latent infection under the epidermis and their subsequent development is arrested till the fruits reach a certain period of senescence. Dastur (1916) showed that *Gloeosporium musarum*, the cause of fruit rot in plantains, established itself much before the fruits are ripe. The lesions of apple scab due to *Venturia inaequalis*, which develop in the stored fruits, are initiated in the field on the tree (Wormald, 1934). Das Gupta and Bhatt (1946) demonstrated five fungi occurring as latent infections in the mango fruit while still borne on tree.

The results obtained from infection experiments on the very young 'Bel' fruits 'in vivo' also go to show to a great extent that the fungi establish as latent infections during the development of the fruits on the tree. This is further substantiated by the presence of these fungi in the atmospheric mycoflora round the 'Bel' tree. They occur nearly throughout the year with variable frequency. Since the atmospheric fungi are likely to change at different places and times, the pathogens coming up as latent infections are also liable to change accordingly from place to place.

The mode of entry of all the causal fungi *viz.*, *B. cinerea*, *A. niger* and *A. nidulans*, in 'Bel' fruits is more or less identical. *B. cinerea* can penetrate successfully through the injured and uninjured stalk end of the young fruits, although the percentage of infection in the former is double than that obtained for the latter, but the aspergilli can do so only if this region is wounded ; and when inoculation is made through the stalk end depression of fruits, *B. cinerea* gains entry only when this is injured while the aspergilli infect through this region even if unwounded. The injured epicarp considerably favours the entry of all the three fungi. After penetration the growth of *B. cinerea* over the tissue is rather slow while the aspergilli grow comparatively faster with the result that they mask the presence of the former when they occur together in older fruits. An infection of *B. cinerea* therefore cannot be detected unless the early stages of development of the infection are taken into account. The presence of *B. cinerea* exclusively in younger fruits and of *A. niger* usually in older fruits either individually or in association with *B. cinerea* may be due to physiological differences in the condition of pulps at these two stages of 'Bel' fruits.

The mechanism and the nature of the latent infection, in this case, is very much similar to that obtained by Das Gupta and Bhatt (1946) who have shown that the infecting hypha remains dormant in the sub-cuticular region till the mango fruits are on the tree and become most active when the fruits are in storage. The dormant mycelium of *Dothiorella ribis* in the air space of the immature avocado pears on trees has been traced by Horne and Palmer (1935), which becomes active in the stored condition of the fruits. In the young 'Bel' fruits, the dormant hyphae have been demonstrated in the sub-cuticular, epidermal and the sub-epidermal regions. A number of spores of *A. niger* have been found attached on the cuticular lining of the fruits, both germinated and as such.

SUMMARY

The paper describes a hitherto unreported dry-rot disease of the 'Bel' (*Aegle marmelos* Correa) fruits in storage.

Three fungi, *Aspergillus niger*, *A. nidulans* and *Botrytis cinerea* have been found to be associated with the disease. Each fungus individually and in combination can cause the dry-rot. The rot produced by *A. niger* is 'black', by *A. nidulans* 'green' while the one caused by *B. cinerea* is 'red'.

The infectional and histopathological studies show that in all the cases the disease is due to the latent infection initiating when the fruits are borne on the tree. The *Botrytis* red-rot appears in very young fruits while the rots due to the Aspergilli develop in mature fruits.

All these fungi gain entry through the stalk end as well as the injured epicarp at all stages of the growth of the fruits.

ACKNOWLEDGMENT

The author expresses his sincerest gratitude and thanks to Prof. S. N. Das Gupta, D. Sc. (Lond.), F. N. I. for his help, guidance and valuable criticism during the progress of the work.

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ON THE NATURE OF THE CELL EXTRUSIONS FROM THE EPITHELIUM IN MIDGUT AND HEPATIC CAECA OF CERTAIN INSECTS.*

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INTRODUCTION

A good amount of work has been done on the structure and activity of the epithelial cells in midgut and hepatic caeca of insects but still there exists a difference of opinion regarding the exact nature of the various cell extrusions observed in the midgut and hepatic caeca of insects. Majority of the workers considered such extrusions as discharged cytoplasmic globules, discharged nuclei, bursting cells, extruded cells etc. to be evidences of the secretory activity and it is on the basis of such an assumption that the discharge of the secretion in midgut and hepatic caeca of insects is described mainly by holocrine and merocrine manners. Some workers however consider these structures to represent cellular degeneration. Still others believe that such structures arise because of poor fixation of tissues. It was with an intention to clear up this confusion that the present study was conducted. Epithelia from the midgut and hepatic caeca of three insects, *Leogryllus bimaculatus* Sauss., *Periplaneta americana* L. and *Gryllodes sigillatus* Walk. were studied under varied experimental conditions.

OBSERVATIONS

Trials with a number of fixatives showed that while fixations by Yao–Nan (Fig. 1) and Mann–Kopsch (Fig. 2) techniques were quite satisfactory, and the condition of the epithelium in these cases was similar to that observed in frozen sections of fresh unfixed material, fixations by Flemming without acetic acid (Fig. 3) and Bouin's fluid (Fig. 4) had a tendency to often produce such structures as discharged cytoplasmic globules, extruded nuclei etc. in large numbers as a result of poor fixation. Flemming without acetic acid also showed an uneven fixation of tissues and the cell extrusions were present in large numbers in regions of poor fixation (Fig. 3). This study thus showed that there did exist a possibility of the production of such extrusions as discharged cytoplasmic globules, extruded nuclei etc. due to poor fixation.

Examination of a large number of epithelia from insects collected from nature showed that there occurs a continuous renewal of the epithelial cells. The old worn-out cells go on degenerating and being extruded out (Fig. 5) and the regenerative cells go on producing new cells which compensate for the loss. This suggested that the few cell extrusions often observed on the top of older cells represented extrusion of old worn-out cells. This study also showed that there occurs, at times, a sudden degeneration and replacement of a part of epithelium and during this condition of sudden degeneration the number of cell discharges becomes quite large (Fig. 6).

*Part of the thesis approved for Ph.D. Degree of Lucknow University.

Fig. 1

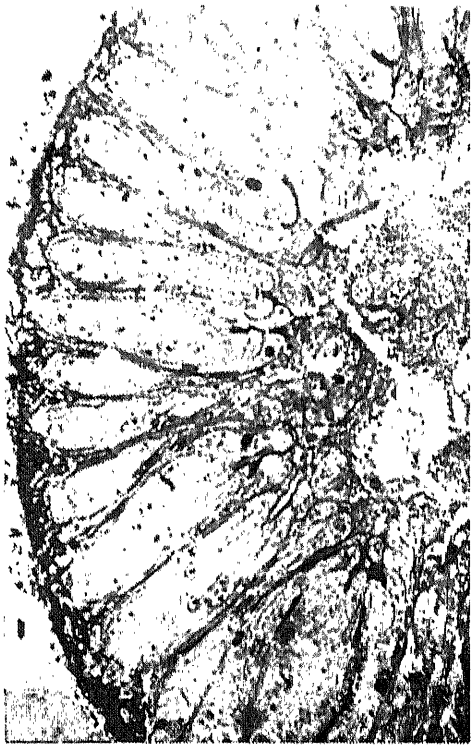

Fig. 4

EXPLANATION OF THE FIGURES

Fig. 1. Photomicrograph of a part of epithelium from hepatic cacca of *L. bimaculatus* Sauss. showing fixation by Yao-Nan fluid.

Fig. 2. Photomicrograph of a part of epithelium from hepatic cacca of *L. bimaculatus* Sauss showing fixation by Mann-Kopsch fluid.

Fig. 3. Photomicrograph of a part of epithelium from hepatic cacca of *G. sigillatus* Walk. showing uneven and poor fixation by Flemming without acetic acid.

Fig. 4. Photomicrograph of a part of epithelium from midgut of *P. americana* Linn. showing poor fixation by Bouin's fluid.

Fig. 5. Part of epithelium from hepatic cacca of *L. bimaculatus* Sauss. showing extrusion of worn out cell. Yao-Nan preparation.

Fig. 6. Photomicrograph of a part of epithelium from hepatic cacca of *L. bimaculatus* Sauss. showing an area where rapid degeneration and extrusion of old worn-out cells is taking place. Mann-Kopsch preparation.

Fig. 7. Photomicrograph of a part of epithelium from midgut (normally fed specimen) of *L. bimacutatus* Sauss showing absence of cell extrusions along epithelial surface. Yao-Nan preparation.

Fig. 8. Photomicrograph of a part of epithelium from midgut of *P. americana* Linn. starved for 2 months. Yao-Nan preparation.

Fig. 9. A part of epithelium from hepatic cacca of *G. sigillatus* Walk. with cells all of equal size. Yao-Nan preparation.

LETTERING

b.b., brush border ; c.m., circular muscles ; ep.c., epithelial cell ; e.ep.c., elongated epithelial cell ; ex.c., extruded cell ; g., granule ; l.m., longitudinal muscles ; n., nucleus ; r.c., regenerative cell ; s.ep.c., short epithelial cell.

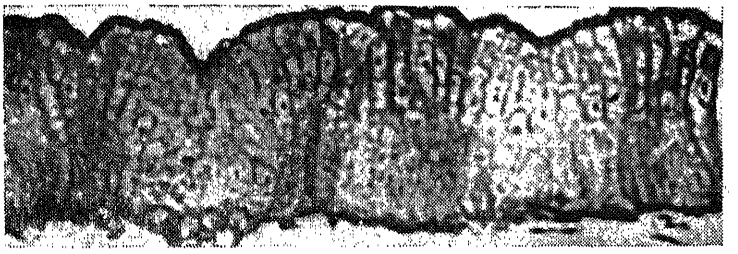

Fig. 2

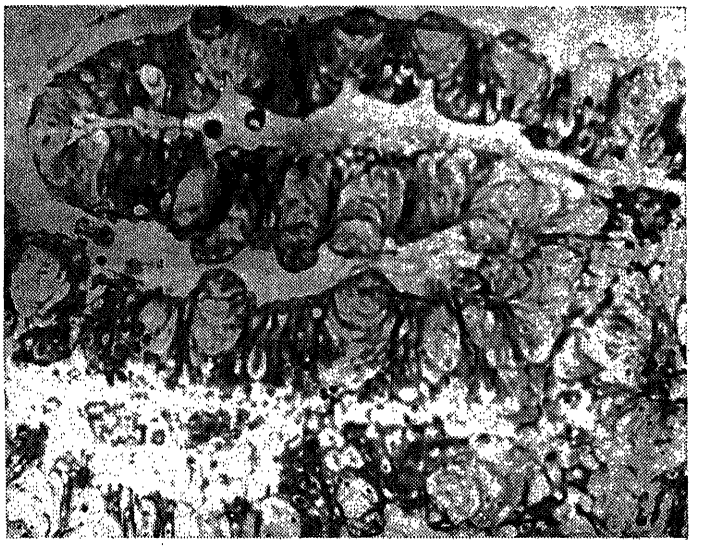

Fig. 3

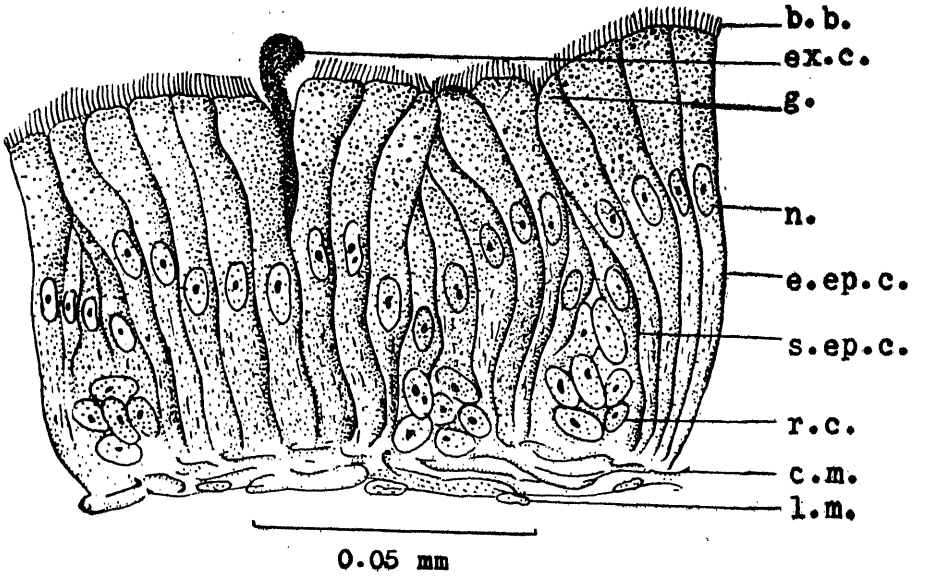

Fig. 5

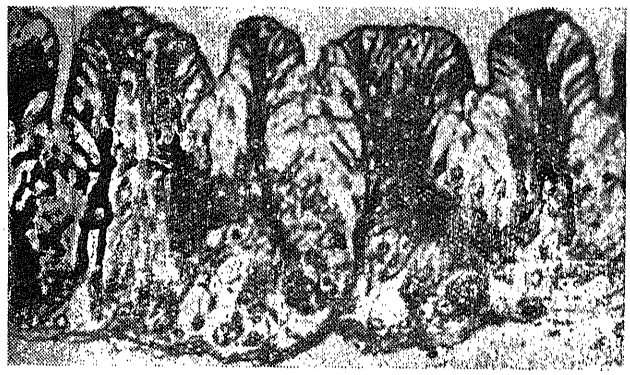

Fig. 7

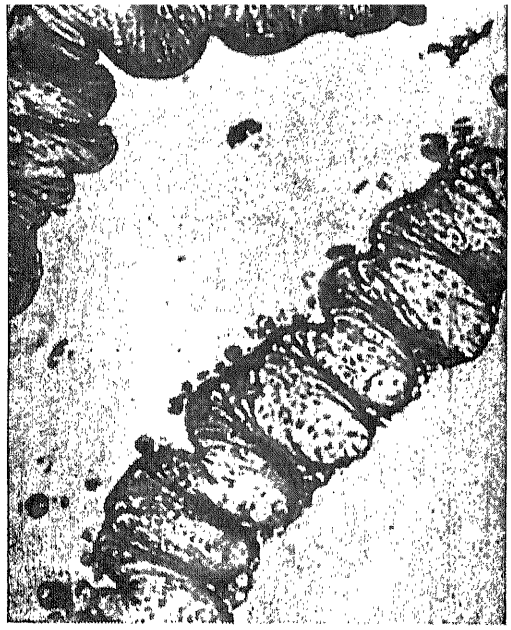

Fig. 6

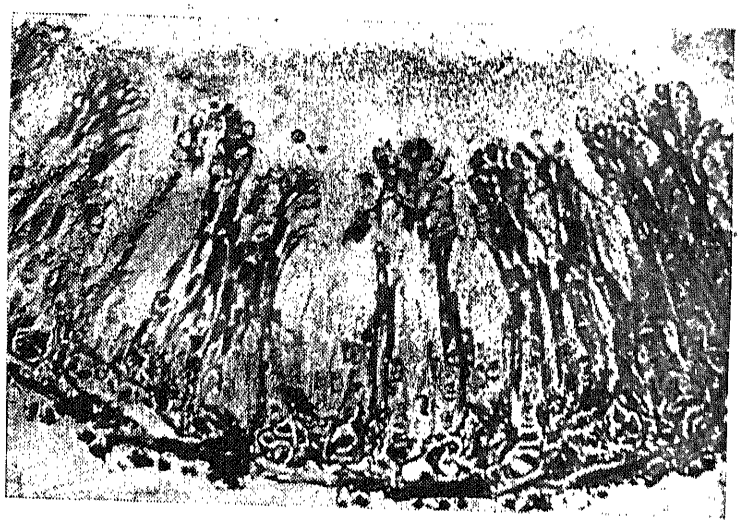

Fig. 8

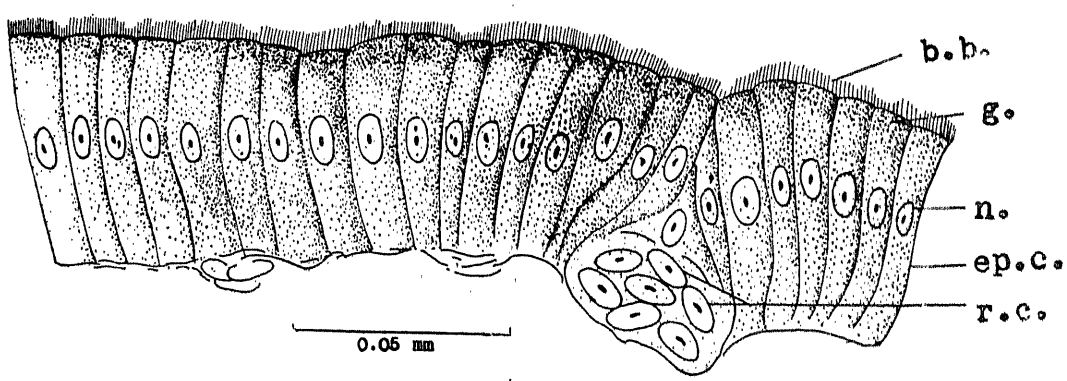

Fig. 9

An examination of a large number of epithelia from insects of the three species under normal feeding condition, unfed condition and under conditions at regular intervals after feeding showed that in majority of the cases the epithelial surface may be devoid of extruding cell discharges and though some extrusions are often observed (Fig. 5), their number is too small to suggest any significance in the matter of secretory activity, these occur on top of old worn-out cells and their presence cannot be coordinated with the secretory activity ; they may be present even in unfed individuals and at times may be absent altogether in some actively feeding forms (Fig. 7) and do not show any increase in their number at any time after feeding. These studies thus clearly showed that these cell extrusions could not be taken to represent secretory discharges.

An examination of epithelia from individuals starved for varied periods showed that even in individuals starved for over two months (Fig. 8) there may be abundance of such discharges along the epithelial surface which clearly showed that these extrusions could not be the secretory discharges, for if they were secretory discharges, there would be no need for their increase in individuals starved for such a prolonged period where there was no food to be digested and so they definitely were result of cellular degeneration.

DISCUSSIONS.

These investigations thus show that the cell extrusions which have been so far generally taken to represent the merocrine or holocrine types of secretory activity are really result of cellular degeneration. A continuous cellular degeneration is going on in the epithelium of insects. The old worn-out cells are being degenerated and extruded out in form of extruded globules or extruded cells. It is, therefore, not strange to see some of these extrusions along the epithelial surface. At times a large number of extrusions may also be seen as there occurs, at times, a sudden degeneration of epithelium. An epithelium (Fig. 9) with cells all of equal size represents a freshly formed epithelium following a rather complete and sudden degeneration. Besides, at times poor fixation may also be responsible to produce an increase in the number of these extrusions. The facts that their presence cannot be coordinated with the secretory activity, these discharges may be present in unfed forms, they may be absent in actively feeding forms, they do not show any increase at any time after feeding, may show an increase during starvation and may be present in exceedingly large numbers in individuals starved for 2 months, afford conclusive evidence that these cell extrusions do not represent secretion discharges and are really result of cellular degeneration. In absence of any visible change in the cells which can be coordinated with the secretory activity, the present author concludes that the discharge of the secretion takes place without any change in appearance of the cells probably by a simple manner of diffusion and the various cell extrusions often observed by the previous workers and regarded as secretory discharges are really result of cellular degeneration.

The view that at times these cell extrusions observed may be result of poor fixation finds support of some previous workers also. Petersen (1912), Steudel (1913), Pavlowsky and Zarin (1922) and Woodruff (1933) stated that the formation of these structures occurs following poor fixation. Similarly continued degeneration and extrusion of epithelial cells and their replacement was described by Henson (1929, 1931) in *Vanessa urticae*, Woodruff (1933) in *Melanoplus differentialis*, Gresson (1934) in *Periplaneta orientalis* L. and Day and Powning (1949) in *Blatella*. A sudden breakdown of epithelium and its regeneration was also observed by Haseman (1910) in *Psychoda alternata*, Newcomer (1914) in beetles, Gresson (1934) in *Periplaneta orientalis* L. and Pradhan (1939) in *Coccinella. septempunctata*. The increase

of the cell extrusions with starvation was recorded by Day and Powning (1949) in *Blatella*. The view that these cell extrusions are not the secretory discharges and the discharge of the secretion takes place without any visible change in appearance of cell finds support by works of only some previous workers. Henson (1931) believed that in *Vanessa urticae* secretion may go on without any change in structure of cells. Green (1931, 1933) in case of *Vespa* had a similar view. Woodruff (1933) in case of *Melanoplus differentialis*, though stated that secretion was discharged in a merocrine manner yet he did not describe discharge of cytoplasmic globules etc. and rather considered these to be due to cellular degeneration. He, therefore, also probably meant that the secretion was discharged without any alteration in the cell structure. Day and Powning (1949) working on *Blatella* clearly showed that the secretion is discharged by the cells in a simple manner of diffusion. More recently Wigglesworth (1953) stated that the secretion in the midgut of mosquito may take place without any alteration in the cells.

A vast majority of other workers, on the other hand, however held, that the cell extrusions as discharged cytoplasmic globules, extruded nuclei etc. represent secretory activity. The earliest classical work concerning these appears to be that by van Gehuchten (1890) on *Ptychoptera contaminata*. He stated that the globular protrusions found on the top of certain epithelial cells were secretory globules. He also mentioned that at times these globules carried nuclei with them. Quite a large number of later workers appear to have taken van Gehuchten's (1890) conclusions for granted and have described various cell discharges as evidences of secretory activity. Poyarkoff (1910) in *Galerucella* and Buchmann (1928) in *Pyrausta* described discharge of secretory globules. Separation of cell tips were described by Cragg (1920) in *Tabanus* and Graham (1934) in *Calliphora*. Gresson (1934) considered in case of *Periplaneta orientalis* L. the separation of cell tips and bursting of cells also as evidences of secretory activity. Hodge (1936) in *Melanoplus* and Saksena (1951) in *Aulacophora* described cytoplasmic globules, extrusion of cells with granules and nuclei, separation of cell tips and bursting of cells as modes of secretory activity.

ACKNOWLEDGMENTS

The author records his grateful thanks to Prof. M. B. Lal, Head of the Zoology Department, Lucknow University, under whose supervision the work has been carried out. To the Government of India the author is indebted for the financial help in form of Govt. of India Senior Research Trainee's scholarship. Thanks are also due to Dr. S. Pradhan for lending some reprints from his personal library.

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ALTERNARIA BLIGHT OF *ZINNIA ELEGANS* JACQ.*

By

PRAKOB KANJANASOON *and* R. S. MATHUR

[Received on 14th February, 1961]

INTRODUCTION

Zinnia elegans, a hardy ornamental plant with variously coloured flowers, is commonly grown in pots or beds almost all the year round in India. But quite often these plants present an ugly appearance in the garden on account of severe leaf spotting caused by Alternaria blight.

Neergaard (1945) inoculated *Alternaria porri* quite successfully on *Zinnia elegans* and a number of vegetables. Baker and Davis (1950) reported *Alternaria zinnia* on a sample of Gerbera from California and *A. Sonchi* on *Zinnia elegans* from Brazil. Kenneth *et al* (1951) found commercial seed fields of *Zinnia elegans* to be severely affected by *A. zinnae.*

Studies reported in this paper are based on collections made by the senior author in the gardens of the Royal Thai Embassy and the Indian Agricultural Research Institute at New Delhi. The affected leaves showed large concentric and often confluent rings which on isolation yielded an *Alternaria* belonging to the *tenuis* group. The morphology, physiology, pathogenicity and host range of this fungus were studied. In addition pot culture experiments, to control the Zinnia leaf spotting by spray fungicides, were also conducted but no field trials could be carried out.

MATERIAL AND METHODS

Alternaria was isolated by sterilizing the affected *Zinnia* leaves for 30 seconds in 1 : 1000 mercuric chloride solution and transferring them aseptically to potato-dextrose agar slants incubated at room temperature A single spore culture was obtained by the dilution method and this culture was used for further studies. This culture was tested for pathogenicity on one month old seedlings grown in 4" diameter pots. Leaves with or without pinpricks were inoculated by a piece of the fungus culture and also by spraying an aqueous suspension of fungus spores on healthy leaves. The morphology of the organism was studied from scrapings of the blight affected *Zinnia elegans* leaves and from the pure culture of the fungus grown on 2% potato-dextrose agar.

The radial growth of the fungus was studied on Richard's agar (Cane sugar-potassium nitrate agar, pH, 6·5) Brown's synthetic medium (glucose asparagine agar, pH, 6·5) and beef extract-peptone nutrient agar, pH 6·5.), oat meal agar pH 6·5 and 2% potato-dextrose-agar adjusted at pH values 4·5, 6·5, and 7·5, according to a technique reported by Cash (1942) and the inoculated petri dishes were incubated in an underground room maintaining a constant temperature of 19–20°C. Final readings were taken on the twelfth day.

In order to study the seed-borne nature of *Alternaria tenuis, zinnia* seeds collected from severely blight affected plants were centrifuged with 5 c. c. of

*Condensed from a portion of the thesis submitted by the senior author for the post-graduate diploma of the Indian Agricultural Research Institute, New Delhi.

distilled water at 800 r.p.m. for about half an hour and after decanting the supernatant liquid, the sediment was examined under a microscope. Surface sterilized seed were also incubated on 2% potato-dextrose agar in petri dishes kept in an underground room maintaining a temperature of 19–20°C.

The effectiveness of a few spray fungicides in controlling *Alternaria tenuis* was further studied on 30 day old pin pricked seedlings which were sprayed with an aqueous solution of hydrophobic colloidal sulphur (1 : 100), Phygon (2, 3-dichloro, 4-napthoquinone, 1 : 1000) and Perenox (50% cuprous oxide, 4 : 1000) before and soon after inoculation by the pathogen. Check plants were inocluated with the pathogen but were not sprayed with the fungicidal solutions. All seedlings were kept in a moist chamber and after seven days, final observations were taken.

All the experiments were repeated three times and each treatment of an experiment was replicated five times.

OBSERVATIONS

Pathogenicity tests carried out according to Koch's postulates by four different methods on 24 leaves showed that the pin pricked leaves of *Zinnia elegans* were the worst affected. (Table 1).

TABLE 1

Pathogenicity tests of *Altenaria tenuis* on *Zinnia elegans leaves.*

Method	Percentage of infected leaves
Pathogen inoculated on pin pricked leaves	66·0
Pathogen inoculated on uninjured leaves·	41·0
Spore suspension of pathogen sprayed on pin pricked leaves	16·0
Spore suspension of pathogen sprayed on uninjured leaves	11·0
Uninoculated control	0·0

The fungus invaded and killed within 5 days portions of the leaf tissue near the pin pricks. There was no sign of fungal invasion on uninoculated leaves. Pin pricked potato and tomato leaves were equally affected. When the affected portions of leaves were surface sterilized and transferred on agar slants, *Alternaria tenuis* was recovered.

The morphology of the causative organism was studied by making a thorough microscopic examination of the scrapings from severely infected leaves. The following observations were recorded :

Mycelium : Light olive, septate, branching 3·04–7·60 μ in breadth.

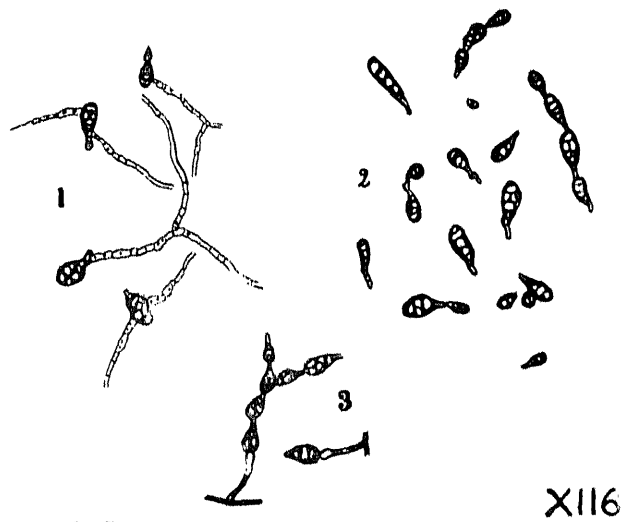
Conidiophores : Light brown, erect, slightly curved or straight, solitary, septate, uniformly thick except at the apex where they are somewhat swollen.

Conidia : Olive brown to buffy brown, oblong or ovate, endochrome, transversely 2–7 septate with longitudinal septae varying from 0 to 4, muriform, 12·54–46·74 μ × 4·90–14·44 μ. The apical cell is short or drawn out into a single cell with short hyaline beak.

Further the morphological characters of a single spore isolation made on potato-dextrose agar showed a variation in spore measurements. The conidia measured 5·70–5·20 μ × 7·60–58·90 μ. Studies of the fungus on an agar slant showed that spores varied a great deal according to the stage of development.

In the thin regions of the slant, conidia were borne in chains of 2 only whereas the thick portions of the slant showed 2–30 conidia in chains.

The cultural characters of the fungus were studied on 7 different media incubated at 19–20°C from the seventh day onwards. Final observations were taken on the twelfth day. The data are presented in figure 1 and table 2.



1. Germinating spores after 24 hrs. in sterilized water.
2. Different shapes of conidia.
3. Conidiophore with chain of conidia.

TABLE 2

Relative linear rate growth of Alternaria sp. on different media

Treatment	Average radial growth of the coloni as in m. m.						Average rate of increase at an interval of 24 hours in m.m.
	7th day	8th day	9th day	10th day	11th day	12th day	
Potato-dextrose agar pH 7·5	19·2	23·5	27·2	31·1	35·5	39·8	4·1
Potato-dextrose agar pH 6·5	24·1	28·0	32·1	36·5	41·4	46·2	4·4
Potato-dextrose agar pH 4·5	18·8	22·0	25·2	28·5	31·6	35·3	3·3
Oat meal agar, pH 6·5	18·0	21·5	25·8	29·5	33·0	37·0	3·8
Brown's Synthetic medium agar, pH 6·5	15·0	18·0	21·1	23·5	26·0	29·2	2·8
Nutrient agar, pH 6·5	15·7	18·3	20·2	22·8	24·7	28·3	2·5
Richard's agar pH 6·5	12·1	14·3	16·5	18·6	20·8	23·0	2·1

The mycelial growth observed on different media was of the following types.

Types of growth observed in media :

(1) *Potato-dextrose agar at pH* 6·5. Shape of colony circular, even, aerial mycelium felt like, Olivacious Black. Growth in uniform concentric rings. Ten rings were formed. Mycelium on the peripheral region colourless. Substratum changed to Dark Olive Gray.

(2) *Potato-dextrose agar at pH* 7·5. Shape of colony circular, even, felt like, Dusky Olive-green. Growth in concentric rings. Concentric ring near the inoculum most conspicuous, after which there is a ring of loose colourless aerial mycelium. Next to the inoculum the growth is depressed. Eight concentric rings were formed. Advancing mycelium on the periphery colourless. Substratum changed to Dark Olive Gray.

(3) *Potato-dextrose agar at pH* 4·5. Shape of colony circular, even, aerial mycelium loose Fluffy-white to Andover Green. Nine concentric rings formed. Substratum changed to Olive Gray.

(4) *Oat meal agar.* Shape of colony circular, uneven, aerial mycelium abundant, convex in growth, thin and short towards the periphery, felt like, Storm Gray. Substratum change to Olive Gray in colour. Concentric rings hazy and inconspicuous.

(5) *Brown's Synthetic medium agar.* Shape of colony irregular, uneven, aerial mycelium very sparse except at the peripherry where it is raised and wholly in texture, Olivacious Black to Dull Greenish Black. Substratum changed to Hathi Gray. No ring formation.

(6) *Nutrient agar.* Shape of colony circular, uneven, aerial mycelium growing like mosses, in clusters and sparse at places Dusky Green Gray in colour, no concentric ring, substrate changed to Dark Olive Gray.

(7) *Richard's agar.* Shape of colony circular, uneven, aerial mycelium very loose, fluffy, sparse, White to Dark Olive Gray in colour. No concentric rings. Substrate changed to Deep Olive Gray in colour.

Potato dextrose agar (pH 6·5 and 7·5) and Oat meal agar, pH 6·5 supported the best growth. The spore measurements were in agreement with Elliot's (1917) description of *Alternaria tenuis.*

An examination of the spore washings from seeds showed rust, smut and Fusarium spores and bits of thick hyphae. Plating of sterilized seed mostly yielded colonies of *Aspergillus* sp. followed by *Penicillium* sp. and *Rhizopus* sp. No conclusions could be drawn whether *Alternaria tenuis* was seed-borne.

CONTROL OF DISEASE

In order to study the effectiveness of some fungicides in inhibiting the growth of the fungus, *Zinnia* seedlings of about 3″ to 5″ height (one month old) were selected, and transplanted at the rate of 5 seedlings per pot of 4″ diameter. They were then sprayed with the following chemicals in the strength given against them.

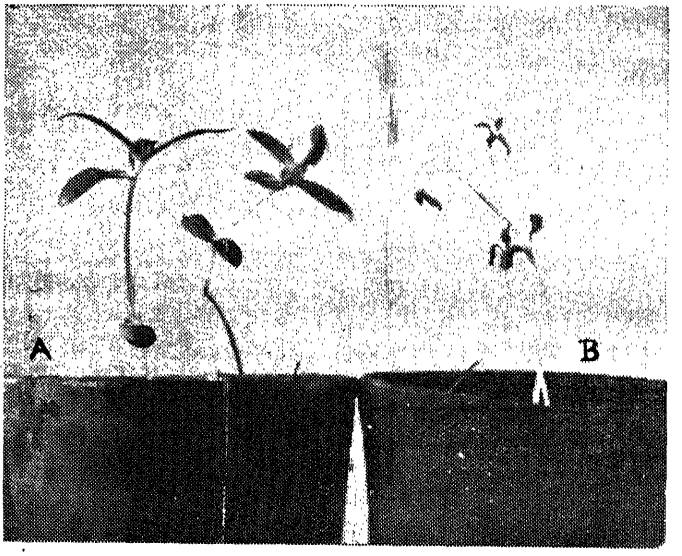
1.	Hydrophobic colloidal sulphur	1 : 100
2.	Phygon (2, 3-dichloro-· 1, 4 naphthequinone)	1 : 1000
3.	Perenox (50% Cuprous oxide)	4 : 1000

N. B. Compared with Color Standard and Color Nomenclature—Robert Ridgway.

Twenty seedlings were used for each treatment with each chemical and another 20 untreated seedlings were kept as control.

Two leaves of each seedling were inoculated with the fungus just after spraying with the chemical. The following method of inoculation was adopted. Pin pricks were made in the centre of each of the leaves with sterilized fine pin, then a bit of mycelium with spores was transferred to this place.

The plants were then kept in moist chamber, the humidity of which was brought nearly to saturation point by frequently spraying the chamber with water with a hand sprayer. Plants were removed after 4 days and kept in the open. The temperature in the moist chamber during the experiment was 28–32°C., and in the open it was 26–30°C. The final observations were taken on the 7th day. The results are given in table 3.



A. Control (Healthy Seedlings).
B. Infected Seedlings.

TABLE 3

Efficacy of different spray fungicides in the control of Alternaria blight of Zinnia elegans

No.	Treatment	No. of leaves inoculated	infected	Percent infection
1.	Hydrophobic colloidal sulphur	40	12	30·0
2.	Phygon	40	5	12·5
3.	Perenox	40	4	10·0
4.	Water (check)	40	24	60 0

It can be noted from these data that spraying with Perenox showed better control of the fungus as compared to spraying with Phygon and Hydrophobic colloidal sulphur. Spraying with Perenox, reduced the percentage of infection to 10·0 whereas in control the infection was 60:0.

In the case of affected leaves, the fungus invaded and killed the tissues surrounding the punctures within 4 days. The affected leaves were collected and surface sterilized with 0·1% mercuric chloride, and transferred to agar slant. After 4 days the same fungus was observed. In the case of control no fungal infection could be observed.

DISCUSSION

In the investigation under report the *Alternaria* causing the blight of Zinnia appeared to differ from *Alternaria sonchi* and *Alternaria zinniae*. The spores were linear to clavate having 2–7 transverse and 0·4 longitudinal septae. The size of spores was 4·99–14·4 μ × 12·54–46·74 μ, and agreed more closely with the measurements reported by Elliott (1917) for the *Alternaria tenuis* group.

Cross inoculation tests indicated that this fungus could infect potato and tomato also. Bhagwagar (1946) described a similar fungus from potato. It is possible that potato and tomato serve as collateral hosts of the fungus.

An examination of the seed washings showed that spores of Fusarium, smut and rust and bits of thick hyaline hyphae were associated with the seed and seedlings raised from unsterilized seed. Seedlings raised from seed protected from external infection did not suffer from *Alternaria* blight. This shows that the *Alternaria* blight common in Delhi gardens is not seed-borne like *Alternaria zinniae* of California. Two sprays of Perenox (4 : 1000) on inoculated leaves reduced the infection from 60 to 10 percent. Preventive sprays on healthy plants but exposed to natural infection of the disease were not tried.

SUMMARY

Symptoms of Alternaria blight have shown large, brown dry areas with concentric rings on leaves.

Pathogenicity tests have shown that the fungus can infect the leaves of the plant by artificial inoculations. The best method of inoculation was transferring the inoculum to pin pricked leaves. The fungus, therefore, appears to be a wound parasite. The fungus is pathogenic both to tomato and potato leaves.

Morphological studies of the causative fungus have shown that it belongs to the *Alternaria tenuis* group.

The fungus was grown on seven different media at 19–20°C. The best growth was obtained on 2% potato-dextrose agar at pH 6·5.

Perpetuation of the fungus on the seeds by the centrifugal and plate methods was studied but *Alternaria* sp. was not isolated.

Perenox spray (0·3 %) on leaves appreciably checked the disease.

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PHYSIOLOGIC VARIATIONS IN *FUSARIUM ORTHOCERAS* APP. AND WR. VAR. *CICERI* PADWICK CAUSING WILT OF GRAM (*CICER ARIETINUM* L.)*

By

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INTRODUCTION

Padwick (1940) studied cultural characters of *Fusarium orthoceras* causing wilt of gram to determine the diagonastic features of the pathogen isolated by him. Cultural variations accompanied with difference in pathogenicity have been observed for a number of species of *Fusarium*. As early as 1913 Wollenweber pointed out such physiologic variations. Broadfoot and Stakman (1926) observed physiological specialization in *Fusarium lini*. Differences in pathogenicity of two strains of the tomato fungus (*Fusarium lycopersici*) was discovered by Haymaker (1928). Watanabe (1939) found variations in toxicity of the cultural filtrates of *Fusarium* spp. In *Fusarium avenaceum* and *Fusarium arthosporioides* variations in cultural characteristic and pathogenicity were found by Cormack (1951). Subramanian (1952) has demonstrated the existence of variations and forms in *Fusarium vasinfectum* causing wilt of cotton. Certain variations in tomato wilt *Fusaria* have also been recorded by Verma (1945).

In view of the wide occurrence of physiologic variations in *Fusarium* it was considered desirable to investigate the twentytwo isolates of the pathogen obtained from wilted plants of gram (*Cicer arietinum* L.) in fields near about Agra and other localities where gram is an important *rabi* crop. Physiologic variations has been studied in respect of (*i*) type of mycelium (*ii*) type of colony (*iii*) radial growth in similar conditions (*iv*) dry weight of mycelium (*v*) toxicity of the filtrate against the host plant and (*vi*) variation in pathogenicity.

METHOD AND MATERIAL

Standard mycological techniques were employed in isolating the pathogen and in cultural studies as outlined by Rawlins (1933). The various isolates were identified following Padwick (1940).

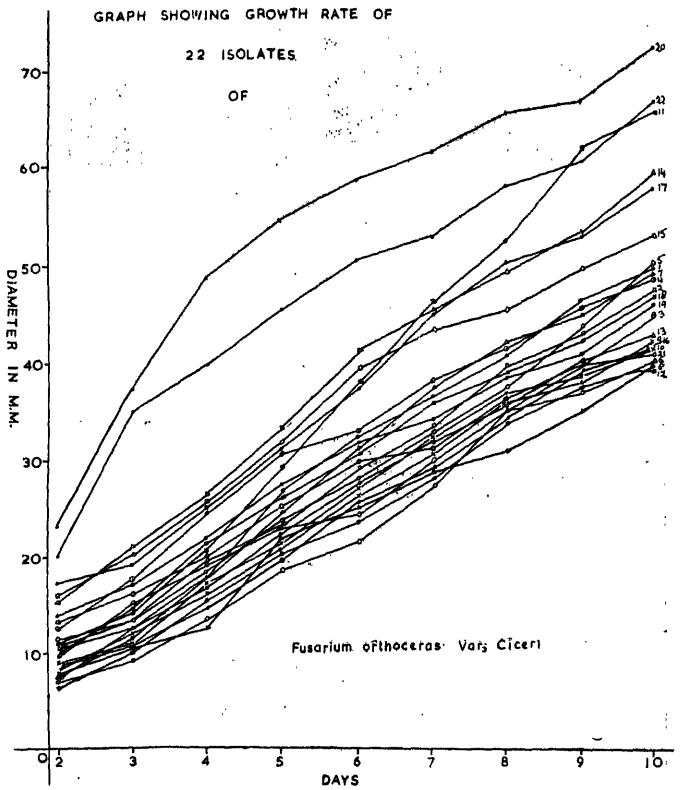
Cultures were grown on Brown's Standard agar medium to note the type of mycelium and monohyphal cultures were developed on Synthetic Czapek's liquid medium to record observations on colony characters. Radial spread was recorded for ten days every 24 hours after two days' growth following Subramanian (1952). The growth relations were also determined quantitatively as suggested by Hasselbring (1908), the weighings were done on a Sartorious single pan balance to maintain the highest degree of accuracy and rapidity. Three replications of each isolate were kept throughout and the cultures were incubated at 28°–30°C.

*A part of thesis approved for Ph.D. Degree of Agra University.

For testing the isolates for the production of toxin they were grown in Richard's liquid medium for 21 days at room temperature, and then the fungus mats were filtered off and the filtrates were tested for the toxin. The seedlings of gram already growing in Shive nutrient solution were transferred to the filtrates of liquid medium in which isolates were previously grown. The time taken (in hours) for complete wilting was recorded. Six seedlings were taken in each treatment (twentytwo filtrates).

The variations in pathogenicity were determined by infesting the soil in pots with the pathogen of different isolates and growing grain seeds of a susceptible variety (T. 87). However, optimum conditions for the disease development were provided as investigated by the author (1959). The final mortality figures in each treatment were recorded.

The statistical analysis of the data was made where it was possible and felt neccassary.



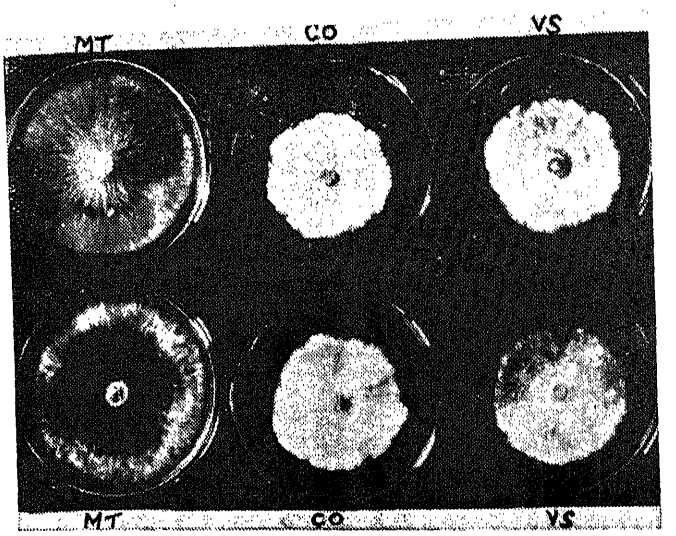
RESULTS

The observations of 22 isolates of *Fusarium orthoceras* var. *ciceri* are summarised. The following symbols for the type of mycelium and colony characters have been used in the following table.

(*i*) Type of mycelium : Cottony CO ; Villous VS ; Matted MT ;

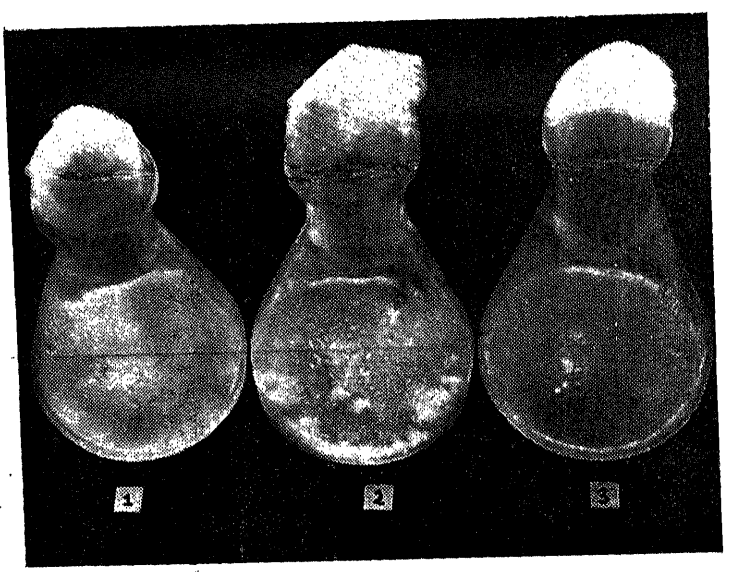
(*ii*) Type of colony : Submerged SU ; Central aerial CA
Aerial mycelium abundant AA

PLATE I



MT : Matted
CO : Cottony
VS : Villous

PLATE II



1. Aerial mycelium abundant
2. Central aerial
3. Submerged

TABLE I

Showing type of mycelium and colony, radial growth (in mm.) and dry weight (in gm.) of mycelium, toxicity of filtrate (time taken in wilting) and percentage mortality in various isolates of *Fusarium orthoceras* var. *ciceri*

Locality of isolate and its number	Type of mycelium	Type of colony	Dry wt. of mycelium	Radial growth of isolates	Time taken in hours	Percentage of mortality
1. Parkham	VS	CA	0·130	45·5	36·0	72·0
2. Raibha	VS	AA	0·168	47·8	35·8	72·0
3. Gwalior (*i*)	CO	CA	0·126	44·6	36·1	73·0
4. Kitham	MT	SU	0·113	48·5	36·1	71·5
5. Fatehpur Chaurasi	CO	AA	0·157	49·9	48·0	60·5
6. Unnao Dubeub	CO	AA	0·119	40·0	72·8	45·0
7. Achhenera	MT	SU	0·137	45·9	36·0	71·0
8. Delhi	VS	CA	0·147	40·0	60·0	50·5
9. Sahjahanpur (*i*)	VS	AA	0·145	41·7	48·1	60·0
10. Sahjahanpur (*ii*)	VS	CA	0·146	41·7	48·0	60·5
11. Kanpur	CO	CA	0·107	65·6	86·0	36·0
12. Lucknow	CO	AA	0·109	39·3	72·1	45·5
13. Unnao Lawain	CO	AA	0·123	42·1	72·1	45·0
14. Etawah	CO	AA	0·146	59·3	60·1	51·0
15. Bichpuri Farm	VS	CA	0·187	53·0	36·1	72·5
16. Gwalior (*ii*)	CO	AA	0·130	42·0	86·0	36·0
17. Fatehpur Matwari	VS	CA	0·153	57·6	48·0	60·0
18. Sikandra	VS	CA	3·198	47·5	48·0	60·5
19. Aligarh	VS	AA	0·157	46·7	60·5	50·5
20. Chheleshar	MT	SU	0·120	72·3	48·0	61·0
21. Khandari Farm	CO	AA	0·179	41·7	59·7	51·0
22. Moradabad	CO	CA	0·165	66·6	86·3	35·5
Critical Difference	..	..	..	4·195	1·25	4·256

Three types of mycelium and three kinds of colony characters can be seen in Plates I and II. It is apparent from the above table that there are differences in the growth rate in some of the isolates both qualitatively and quantitatively. The differences in the radial growth can also be made from the graph.

The most interesting relationship which is revealed from the above table is between toxicity of the filtrates and their pathogenicity. One is directly proportional to the other. With increase in the time of wilting in the filtrate the percentage mortality also decreases correspondingly. On the basis of toxicity

of the filtrates and also the percentage mortality five groupings of the isolates are formed. These groupings are very distinct from each other (C. D. 1·25 and 4·256 respectively) and are as follows :

TABLE II

	NUMBER OF ISOLATES					
Group (*i*)	1	2	3	4	7	15
Group (*ii*)	5	9	10	18	20	17
Group (*iii*)	8	14	19	21		
Group (*iv*)	6	12	13			
Group (*v*)	11	16	22			

The number of isolates in various groups are the same both in respect to toxin production and the pathogenicity. There is no posibility of mixing the two isolates of different groups in one, as the figures are quite high in comparison to the critical differences (Table I).

Table I also reveals that there is no correlation between the amount of toxin production and the rate of growth in artificial media. However, there is a significant correlation between toxicity of the filtrates and percentage mortality as shown by groupings.

DISCUSSION

A study of the data presented shows that physiologic variations exists within the species of *Fusarium orthoceras* var. *ciceri*. Tests for pathogenicity, toxin production and cultural characters have shown distinct differences among 22 isolates studied. The isolates range from apparently mild to high toxin producing and pathogenic types under controlled conditions. In pure cultures distinct differences were found in a number of isolants. The variations in toxicity of the filtrates, pathogenicity and in the rate of growth are sufficient to delimit the isolates in distinct groupings. However, no attempt has been made to classify any given isolant as a permanent strain.

The establishment of strains of the fungus studied is complicated by dissociation, a phenomenon common in most fungi (Das Gupta 1936). Because of this the stability of any single isolant or strain cannot be considered permanently dependable. Changes may Suddenly take place in the morphology, physiology or pathogenicity of an apparently stable strain (Bailey 1934). Consequently, the establishment and identification of strains is not feasible, except for the duration of the period of study. Nevertheless, this does not lessen the probability of picking up isolants with varying degree of virulence or possessing other variable characters from fields.

Any attempt to establish a permanent strain of a *Fusarium* species is likely to meet with disappointment, for the permanency of a strain or isolant may continue for several years or it is short-lived (Leonian 1932). The possibility that dissociation occurs in the soil, thereby giving rise to new or distinct strains, has been pointed out earlier. This phase of dissociation which has been suggested explains why isolants from diverse localities or even from the same locality may vary in toxin production, pathogenicity or other characteristics. It is strongly felt that dissociation occuring in the soil is responsible for the appearance of many variable isolants or strains.

Since dissociation in the soil must be of frequent occurrence and the direction in which the variation may take is a matter of chance, the number of distinct strains (degree of physiologic specialization) appears to be quite indefinite (Wellman 1943).

It has been noticed that there is no correlation between the amount of toxin production (toxicity of the filtrate) and the rate of growth in the artificial media but there is a significant correlation with the percentage mortality indicating toxin role in the mechanism of wilting, Chauhan (1959).

SUMMARY

Physiologic variations of the pathogen (*Fusarium orthoceras* App. and Wr. var. *ciceri* Padwick) in 22 isolates from localities near about Agra and elsewhere, where gram (*Cicer arietinum* L.) is an important *rabi* crop were recorded on the basis of type of mycelium and colony characters, linear growth, dry weight of the fungus mat, toxin production and pathogenicity. Three types of mycelium and the same number of colony types were detected among the isolates. On the basis of linear growth a few groupings within these isolates were found. The toxin produced by the different culture filtrates showed varying degree of toxicity. These isolates also showed variations in pathogenicity. No correlation has been obtained in relation to vigour of the pathogen and the toxicity of the toxin produced by them, however, a significant correlation was there, between toxicity of the filtrates and their pathogenicity. Five common groupings were obtained for the toxicity of the filtrates and percentage mortality in pot-cultures.

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*Original not seen.

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